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SOME INVESTIGATIONS ON ENTOZOIC PROTOZOA¹

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FIVE years ago a School of Hygiene and Public Health was established as a part of the Johns Hopkins University. One of the divisions of this new school is a department of medical zoology. This is the first department of the kind to be organized in this country, and it has been necessary on this account to determine what actually constitutes medical zoology and what phases of the subject can appropriately be taught and investigated in an institution devoted to preventive medicine. This evening I wish to say a few words regarding medical zoology as a subject and then describe briefly some of the investigations we have carried on in the department on entozoic protozoa.

At the present time the department consists of four divisions, devoted, respectively, to protozoology, helminthology, medical entomology and the filterable viruses. The filterable viruses are agents of disease about which we know very little. They are included but tentatively in the field of medical zoology until we know more definitely what their real status is. The term medical entomology does not exactly indicate the field covered by this subject, inasmuch as not only insects but other arthropods, such as mites and ticks, are included. From the vast assemblage of arthropods those particular species are se-

¹ A lecture delivered at the Marine Biological Laboratory, Woods Hole, Mass., August 3, 1923.

lected that serve as vectors of disease-producing organisms or are actual parasites themselves. Helminthology is likewise a subject that includes forms selected from various groups of animals, especially flatworms and roundworms, that are of medical or veterinary interest. Among the more important of the parasitic worms from the standpoint of public health are the several species of hookworms, flukes, ascarids and filariae.

The teaching of medical zoology differs in no essential feature from that of general zoology. The material to be taught must be selected just as material is selected for the teaching of embryology, invertebrate zoology or physiology, and similar methods are used in presenting the subject-matter to students. Investigations in medical zoology are also similar to those carried on in other fields. They, however, not only involve fundamental biological problems but also frequently have a more or less intimate relation to preventive and curative medicine. It is, therefore, not unusual for a medical zoologist to see the results of his investigations translated almost immediately into terms of human welfare.

Often the terms medical zoology and parasitology are used synonymously. Most of the arthropods of medical importance, however, are not parasites, but are transmitters of disease-producing organisms such as bacteria, protozoa and filariae, and a large proportion of the protozoa referred to in books on parasitology are entozoic but not parasitic. It seems best, therefore, to substitute the term medical zoology for the more common term parasitology.

Many of the most complete accounts of medical zoology are to be found in reference books on tropical medicine, and often as much as one half of such books is devoted to this subject. Much of this space is given over to a zoological treatment of the organisms discussed, thus emphasizing the relative importance of this phase of the subject. We are accustomed to think of many of the diseases that are caused by animal parasites as tropical in their distribution, and it is true that temperature condi-

tions and lack of sanitation make the tropics a favorable environment for the multiplication and spread of these organisms and thus lead to mass infections that bring about the appearance of clinical symptoms. But many of these diseases were once prevalent in temperate regions and even now exist to a certain extent in colder portions of the earth. Their almost complete absence from these regions is largely due to the application of our knowledge of prevention. Some of the most conspicuous successes in the field of preventive medicine have resulted from attacks on diseases due to animal parasites in tropical countries, these organisms lending themselves more readily to preventive measures than most of the other agents of disease.

The protozoa have been favorable material for investigation ever since their discovery, and many of the greatest figures in the history of biology devoted their time largely to this group of organisms. At first free-living species were widely studied and monographed, and only comparatively recently have entozoic forms attracted particular attention. This has resulted from the discovery that certain diseases of man and of lower animals are due to the presence of pathogenic protozoa. Medical men as well as zoologists have carried on investigations on these entozoic species, and medical literature as well as the literature of zoology is in consequence abundantly supplied with the results of these researches. Because of their economic importance certain species are emphasized and others of equal importance, biologically, neglected. However, in almost every case what we know about the species pathogenic to man is based, first, on our knowledge of free-living forms, and second, on related species that occur in lower animals.

In the School of Hygiene and Public Health we consider it appropriate to carry on investigations on all the three types of protozoa just mentioned, namely, free-living species, species entozoic in lower animals and species living in man. Our attention, however, is particularly directed to the study of the entozoic forms and I

shall now attempt to present briefly some of the results of our investigations with these organisms.

For the sake of convenience, we can separate the forms to which I wish to refer into blood-inhabiting species and intestinal inhabitants. Among the most interesting of the former are the malarial parasites and the trypanosomes. The methods of malarial control are comparatively simple and well known, but many important problems still remain to be solved. Of these, one of the most interesting is the problem of relapse. A person who has been infected with malarial organisms by the bite of a mosquito, experiences symptoms as soon as a sufficient number of organisms become present in the blood as a result of asexual multiplication. If the resistance of the host is not sufficient to prevent continued multiplication of the organism, death eventually results. If, however, the patient recovers either with or without treatment, he usually still harbors parasites within his body, which may at some later date again become so numerous as to bring about the recurrence of symptoms, a condition known as a relapse. There are various theories to account for relapses in malaria, one of which we believe we have finally proved. It is difficult to study the subject in the human host; but malarial organisms similar to those in man also occur in birds and can be cultivated in canaries with ease. Drs. Whitmore and Ben-Harel have spent several years investigating the phenomena of relapse in these birds. Apparently in most cases a bird when once infected remains infected throughout its entire life. Clean birds are infected by injecting a small amount of blood from a diseased bird into the breast muscle or peritoneal cavity. Parasites begin to appear in the peripheral blood within about two weeks. They increase rapidly in numbers for a few days and then as rapidly decrease until none can be discovered even after very careful search over a long period of time. The blood of such a bird, however, is still infective to clean birds, and Whitmore was able to inoculate successfully clean birds with the blood of a canary 29 months after the original infec-

tion. It is evident that organisms in some stage in their life history are present in the peripheral blood during this entire period. They may be asexual stages that are too few in number to be found in ordinary preparations, or there may be a stage still unknown in the life history of the organism that has not been brought out by our present method of study. In what part of the host the parasites are located during the intervals between relapses and in what stage of their life cycle are questions that have recently been answered by the researches of Dr. Ben-Harel. She has found that during the period when parasites are apparently absent from the blood, they exist in small numbers in the spleen and bone marrow where they undergo asexual reproduction in a normal fashion. Presumably asexual reproduction in certain of the internal organs proceeds throughout the interval between relapses, the young parasites being liberated from time to time in small numbers into the blood. Conditions favorable to the organism may result in a sudden rapid increase in their numbers, thus bringing about the clinical symptoms which we know as a relapse. With these facts thus well established it is possible to conduct experiments with therapeutic agents designed to attack the parasites that are located in the spleen and bone marrow and are apparently immune to attack by quinine as now employed as a preventive and curative agent. Professor Boyd is now at work on this problem, using the organisms of bird malaria as they exist in canaries and subjecting them to various quinine derivatives kindly prepared for us by Dr. Jacobs of the Rockefeller Institute for Medical Research.

Other problems in malaria that are being investigated by members of the Department of Medical Zoology of the School of Hygiene and Public Health concern the relation between the parasite and its mosquito host. Before any malarial campaign can be carried on with success, we must determine by experiment what particular species of anopheline mosquito acts as a transmitting agent in each particular locality. We must determine what we may

call the biology of these species in order that effective methods of elimination can be carried on. These and other problems of mosquito control are now being investigated at Leesburg, Georgia, by Drs. Darling and Root.

The second group of blood-inhabiting protozoa of great medical importance contains the trypanosomes. The trypanosomes are responsible for the two types of African sleeping sickness, for Chagas's disease, which is prevalent in certain regions of South America, and for diseases in various domesticated animals throughout the world. Studies have been carried on in our laboratory with trypanosomes of frogs, salamanders, rats, man and various domesticated animals, and with several of their intermediate hosts. The first trypanosomes ever described were discovered in fish. Soon after this discovery the frog was found to be parasitized by this type of protozoon. But it was not until these organisms were known to be the causative agents of diseases of domestic animals and later of man that they really came into prominence as objects for research. Our investigations have been concerned principally with the genetics of these organisms and the resistance set up by the host to experimental infections. *Trypanosoma diemyctyli* that occurs in the common newt, *Diemyctylus viridescens*, was the first form studied by us. All the organisms present in the newt, at least in the month of May, appeared to be adults. Of particular interest was the discovery that constant differences occurred in the length of the trypanosomes occurring in different hosts. It seems probable that the trypanosomes from different hosts represent races that are heritably diverse in size, although this difference may be due to differences in the environment as represented by the blood stream of the different hosts.

More elaborate investigations have been carried on by Dr. Taliaferro with trypanosomes in rats. Pure lines of this species, *T. lewisi*, were obtained by inoculating clean rats with single trypanosomes. Infections started in this way indicate that reproduction of the trypanosomes in the blood of the rat ceases within 25 days after the or-

ganisms begin to appear in the blood. From this time on, practically no further reproduction or growth occur and only adult trypanosomes are present. On the basis of these observations an attempt was made to determine whether growing the same pure line in host rats of the same species results in significant differences in size, and whether these differences are similar to those obtained when grown in different species of rats. A statistical study of the data indicates that significant differences of about the same magnitude occur in both cases, but whether these differences are due to heritable diversities or to differences in environment has not been determined.

Another result obtained from these data is that there is no significant change in the coefficient of variation when the pure line is passed from rat to rat by blood inoculation. In contrast to this is the fact that the pure line which does not change in variability when passed through rats increases in variability when passed through its natural insect vectors, the rat fleas. The results indicate that the pure line breaks up during its passage through the invertebrate host, due probably to some nuclear phenomena which may be either the result of a sexual process or of reorganization. No processes, however, such as conjugation or endomixis, have yet been demonstrated in the life cycle of the trypanosome.

As an outcome of this genetical work, Dr. Taliaferro has undertaken an intensive investigation of the resistance which the rat offers against an infection with *T. lewisi*. He finds that there are three manifestations of such a resistance: (1) The rate of reproduction of the parasites is retarded more and more until it is finally inhibited altogether by about the tenth day; (2) a large number of parasites are destroyed between the tenth and fourteenth days; (3) all the rest of the trypanosomes, which exist in the blood as non-reproducing adults, are destroyed from a week to several months afterward (end of infection). The differentiation between that type of resistance which inhibits reproduction and that type which destroys the organisms after they are formed was

made possible by a new method of measuring the rate of reproduction which is irrespective of the number of organisms destroyed. The method consists essentially in comparing the coefficient of variation for some factor involving size and is based on the well-known fact that a sample taken on one hand from a population undergoing rapid reproduction, with the constant production of young forms and intermediate growth stages, will exhibit much greater variability in size than a sample taken on the other hand from a population in which there is little or no reproduction and in which all the organisms are full-grown adults. Results now in press demonstrate that the first manifestation of resistance (the inhibition of reproduction, mentioned above) is brought about by the formation in the blood of infected rats of a reaction product which has the specific property of inhibiting reproduction in the trypanosomes but which does not affect their vitality. This reaction product differs from those known at the present time. To date no mechanism of the second effect of resistance, *viz.*, the first drop in numbers, has been ascertained, but preliminary experiments on the third effect, *viz.*, the total destruction of the organisms, indicate that it is brought about by the formation of a lysin. Work with various pathogenic trypanosomes in different hosts indicates that although, in some, there seems to be a manifestation of resistance similar to the second effect in *T. lewisi*, there is never any inhibition of reproduction.

The second large group of entozoic protozoa to which we have directed our attention contains inhabitants of the intestine of man and of lower animals, and includes genera of flagellates, ciliates and amoebae. These organisms can be studied as they occur in their hosts, but it always facilitates investigation to be able to cultivate the species, with which we are working, outside of the body, especially when the investigation involves experimental studies.

Several common intestinal protozoa have been cultivated for the first time in our laboratory. *Chilomastix*

mesnili, which is a flagellate often associated with intestinal disturbances and which is present in about 4 per cent. of all human beings, was obtained in cultures by Dr. Boeck and could apparently be transferred from one culture tube to another indefinitely. A second species of human intestinal flagellate that has been cultivated for the first time is *Embadomonas intestinalis*. Dr. Hogue succeeded in rearing this species on simple culture media and found that encystment took place under cultural conditions, this being the only species of human intestinal flagellate thus far recorded that encysts in cultures. A third species of intestinal flagellate from man, *Trichomonas hominis*, may likewise be cultivated in simple media. We have on a number of occasions obtained pure lines of this form by inoculating a tube with a single specimen. Trichomonads may possess three, four or five flagella, but when a pure line is obtained all the progeny are provided with the same number of flagella as the original progenitor, indicating that the number of flagella is a heritable character.

The perfection of methods of cultivation has made it possible to undertake investigations of several types. In the first place it provides a more practical and efficient method of diagnosis. The usual procedure in making a diagnosis is to prepare a smear of the suspected fecal material and examine this for flagellates. Dr. Becker and I have found, however, that placing a similar fecal specimen into a culture tube where it is allowed to remain for several days before examination is a more certain method of detecting the presence of these organisms. The perfection of this method will in the future enable us to make more accurate surveys of intestinal protozoa. The effects of external factors on intestinal protozoa can also be determined by studying the results of modification of the culture medium, and work of this sort is now in progress.

With the exception of *Trichomonas hominis*, the various protozoa mentioned are known to form cysts which serve to maintain the race during the period of transfer

from one host to another and to withstand the secretions of the alimentary canal during the passage from the mouth to the intestine of new hosts. From the standpoint of prevention it is important to know something about the longevity of these cysts under various environmental conditions and to determine in what way cysts are disseminated. Dr. Boeck has shown that all the environmental conditions that the cysts are apt to encounter, except drying, can be met successfully by them, but that cysts live for a greater length of time when they are free from the bacterial mass in which they are embedded. Liquids such as water and milk are thus shown to be especially favorable for their distribution. It is generally supposed that cysts find their way from one host to another in drinking water and food that is contaminated and that this contamination is often accomplished by house flies. Dr. Root has been able to prove by a long and careful series of experiments that cysts are easily taken into the alimentary canal of house flies and pass through the body of the fly without injury. It is thus possible for flies to become contaminated and to spread this contamination to food on which they may chance to alight. There can be no doubt that this actually does happen in nature.

Conditions prevailing during the late war stimulated a widespread interest in intestinal protozoa, and besides adding to our knowledge several species that were hitherto unknown, gave us some idea of the extent of the infection with these organisms among human beings. Dr. Payne and I compiled statistics from over 35 papers published by American, English and French investigators, which recorded data from about 20,000 persons. Twenty per cent. of these were infected with intestinal amoebae of the species *Endamoeba coli*, 9 per cent. with *Endamoeba histolytica*, 12 per cent. with the intestinal flagellate, *Giardia lamblia*, 4 per cent. with *Chilomastix mcconnili*, and 3 per cent. with *Trichomonas hominis*. So high is the percentage of infection that we usually have no trouble in obtaining material for study or research from some member of the department. It is

probable, even, that among those who are here in this room this evening, there are perhaps 60 infected with *Endamoeba coli*, 30 with *Endamoeba histolytica*, 35 with *Giardia intestinalis*, 12 with *Chilomastix mesnili* and 9 with *Trichomonas hominis*. Fortunately, these organisms are not usually pathogenic or at least the results of their presence are so slight that clinical symptoms do not appear unless enormously large numbers are present.

What effect the changes in the diet of the host might have on the incidence, distribution and numbers of these intestinal protozoa is a question that I have attempted to answer on the basis of experimental work with tadpoles and rats. Particular attention was at first directed toward the problem as presented by the opalinid ciliates in the green frog. These opalinids, at least in certain localities, occur in 100 per cent. of green frog tadpoles but are absent from the adults. This is in contrast to the condition that exists in certain other Anura, for example, in the leopard frog and the common toad. In these forms the infection with opalinids is continuous from tadpole to adult. Dr. Metcalf has suggested that the disappearance of these ciliates in the case of the green frog might be due to the change from a vegetable to an animal diet. Tadpoles, however, that were kept in the laboratory and fed on a strict animal diet did not lose their opalinids until the time of metamorphosis. During metamorphosis not only these experimentally-fed tadpoles but controls collected in the field also lost their opalinids. Apparently, therefore, the change from a vegetable to an animal diet is not the controlling factor. It has been known for many years that metamorphosis in tadpoles is accelerated when thyroid gland material is used as food, and there is some evidence that the presence of some substance in the thyroid may accelerate growth and asexual multiplication in certain free-living ciliates. No increase in the number of opalinids, however, was found to occur in tadpoles of the green frog when fed on thyroid substance, but metamorphosis was hastened and as metamorphosis proceeded, the opalinids

disappeared. It thus seems certain that changes in the digestive tract of the green frog at the time of metamorphosis are responsible for the loss of opalinids and not changes in the diet of the host and that thyroid substance does not accelerate the growth and division of these organisms as it has been supposed to do in free-living species. Why reinfection of the adult green frog does not occur is still unknown.

The intestinal protozoa of the rat to which particular attention was directed are *Giardia muris*, *Hexamitus muris* and *Trichomonas muris*. I shall refer this evening to the third named species only, *Trichomonas muris*. This species is usually present in great abundance in the cecum of the rat, the material of the cecum frequently consisting almost entirely of a wriggling mass of these organisms. Among a large number of rats obtained from the rat colony of Dr. McCollum that had been fed on various experimental diets, three were found that were free from all intestinal flagellates. The history of these three rats showed that both they and their parents had been fed exclusively on a carnivorous diet. Experiments were initiated on the basis of this discovery that gave striking results in a remarkably short period. Control rats from the colony maintained by the Department of Medical Zoology were found to be 100 per cent. infected with *Trichomonas muris*. These rats had been fed on a diet largely vegetable in nature. Twenty rats from this colony were fed on a well-balanced carnivorous diet for one week and were then sacrificed and examined. Only one of these rats seemed to be entirely free from *Trichomonas muris*, but the number of organisms decreased almost to the point of extermination in the other nineteen. The method of counting used was to take a measured amount of material from the cecum, dilute it with a measured amount of normal saline solution, spread this out under a cover glass 22 mm square, and then count the average number of organisms in ten fields, using a number 10 ocular and a 16 mm objective. It was found by this method that the average number of specimens

of *Trichomonas muris* per field in control rats was 90, whereas an average of less than 2 per field appeared in the rats fed on a carnivorous diet for one week. These results indicate that a carnivorous diet brings about a change in the environment within the cecum of the rat that is very unfavorable to the flagellates. Studies of the hydrogen-ion concentration of cecal material in experimental and control rats indicate that changes of this nature are too slight to account for the results. The rat has been a favorable object for the study of the effects of various diets, and at the present time the explanation that seems best to fit the results obtained by my experiments is based on the character of the bacteria and of the products of their activity. Cannon, for example, has recently shown that when rats are fed on a vegetable diet, intestinal bacteria are present in the ratio of about one of the colon type of 99 of the acidophilus type, and that when a carnivorous diet is substituted for the vegetable diet, this ratio is reversed within a few days to about 99 of the colon type to one of the acidophilus type. The sudden change from a preponderance of the acidophilus type of bacteria to a preponderance of the colon type seems, at present, to be the principal cause of the disappearance of the flagellates. Since these experiments were completed, I have been in communication with three investigators who have carried on similar work with the intestinal protozoa of rats and whose results confirm mine.

Flagellate diarrhoea is a very obstinate disease which often leads to conditions that result fatally. No successful treatment has ever been devised for its alleviation. These experiments on rats, however, suggest that a carnivorous diet for a short period might be an effective method of treatment. This method has actually been put into practice at one of the largest clinics in this country and although up to the present time only a few cases have been available, the effects of the treatment have been remarkably rapid and satisfactory. Inasmuch as one in-

investigator found that the amoeba of the rat are adversely affected by feeding the host on a carnivorous diet, it seems probable that amoebic dysentery in man may also be successfully treated by proper changes in the diet.

One of the most complex of all biological relationships is that between an entozoic species and its host, and many efforts have been made to determine the evolutionary stages that have ended in parasitism. Various types of association between animals are known. Certain of these involve temporary associations that may even be unnecessary for the successful fulfillment of the complete life cycle of the organism. Other associations exist that are temporary but necessary, and from this point on all stages of association are known ending in a fixed parasitic existence. Many students of parasitic animals have speculated regarding the various stages in the evolution of the organisms in which they were primarily interested. While these speculations are legitimate, the conclusions reached can be of very little value without more thorough observation and experiment as a basis. In contrast to these speculations are the painstaking investigations, such as those on the opalinids recently published by Metcalf, which bring together a vast fund of information, enabling the investigator to reach conclusions based on facts.

Recently an association came to my attention which promises to be favorable for the study of the evolution of parasitism. On a number of occasions during the past decade, I have observed living flagellates of the euglena type in the intestinal and rectal contents of frog tadpoles. They were always considered merely accidental visitors that had been ingested with the food of the tadpoles and were either immune to the digestive juices and were on their way through the intestine, or had not yet succumbed to digestion. Last year, however, these organisms were found in such abundance in certain tadpoles that it occurred to me they might be normal inhabitants of the intestinal tract of these animals. It was first discovered that they are different from any

other members of the Euglenoidina, in that they possess three flagella instead of the usual one or two. It was then proved that they are not digested by the tadpoles nor simply on their way through the alimentary canal, since tadpoles that were starved for 30 days continued to retain in a flourishing condition apparently every specimen originally present. Tadpoles of the green frog, leopard frog and toad were found to be infected, and various experiments proved that uninfected tadpoles of any one of these species could be infected by feeding them active trophozoites from the intestine of either of the other two species. Apparently these euglena-like entozoic flagellates are non-specific with respect to these three species of hosts. A striking feature of these organisms is their retention of green chromatophores and red eye-spots while living within the digestive tract of the tadpole. It was found, however, that the color of both chromatophores and eye-spots became somewhat faded in specimens living in the intestines of toad tadpoles. Apparently sufficient light passes through the semi-transparent body wall and intestinal wall of the tadpoles of the green frog and leopard frog to insure the maintenance of the color, whereas the extensive pigment in the body wall of the toad tadpole prevents the entrance of light. By a curious coincidence Dr. Wenrich began a study of these organisms at about the same time that I did. His time was devoted largely to their cultivation and division and his studies indicate that there are two varieties, one green and the other colorless or nearly so and that the green form may transform into the colorless one.

In order to determine whether other types of Euglenoidina could maintain their existence in the digestive tract of the tadpole, I fed uninfected animals on several types of free-living euglenae. The first type used was a small species possessing two short flagella. Millions of these were devoured by the tadpoles, every one of which quickly disintegrated in the tadpoles' intestines. In a second experiment, large reddish-colored euglenoids were

fed to tadpoles of the green frog. Specimens of this species were able to withstand the conditions within the intestine, many of them passing through the digestive tract apparently uninjured; but none of them remained in the intestine or rectum more than 24 hours. A third type of euglenoid fed to tadpoles was discovered by Dr. Reynolds living in the bladders of the bladder-wort, *Utricularia*. Three tadpoles of the green frog were fed 20,000 of these euglenoids; all the euglenoids were immediately destroyed within the intestine. It is thus evident that although this type of euglenoid is able to resist the secretions within the bladder of the *Utricularia* plant, it can not withstand the digestive juices of the tadpole. Several parasitic species of *Euglenoidina* have been recorded in the literature. For example, there is a species of *Astasia* that lives in the digestive tract of a fresh-water nematode and one that lives in the digestive tract of *Cyclops*. It seems probable that a large number of *Euglenoidina* may be more or less parasitic in habit and that considerable light may be thrown on the evolution of parasitism in this group by further studies of the relations between free-living and entozoic species.

As already indicated, the entozoic euglenoids of tadpoles seem to be non-specific with respect to their hosts. This probably indicates a recent adoption of the entozoic habit. This condition of non-specificity of host is also illustrated by certain other intestinal flagellates, notably the herpetomonads that live in the intestine of certain flies. Dr. Becker has shown that the herpetomonads of six species of muscoid flies, which have up to the present time been considered distinct species, are morphologically identical. Specific rank for these herpetomonads must therefore be based on differences of host and not on morphological characteristics. However, Dr. Becker has been able to show by cross-infection experiments that the herpetomonads from any one of the six species of flies will infect clean flies of any of the other species. There is thus shown to be no evidence that specificity of the parasite can be based on the character of the host in this

group of flagellates, and we must conclude that these six species of flagellates in reality all belong to one species.

In contrast to the condition observed in the case of these herpetomonads may be cited the observations and experiments of Dr. Simon and myself on intestinal flagellates of the genus *Giardia*. *Giardia intestinalis* is one of the commonest intestinal protozoa of man, being present as previously stated in about 12 per cent. of all human beings. It is accused of being responsible for intestinal disturbances, although this has not definitely been proved. For many years the giardias found in rats, mice and several types of domestic animals were supposed to be one and the same species, and the contamination of the food of man by cysts from rats and mice was supposed to be the common method of human infection. In 1908 Benson recognized three species of giardias, one from man, one from the rabbit and one from rats and mice. A fourth species was described by Kofoed and Christiansen in 1915 from the field mouse. The careful statistical and cross-infection experiments of Dr. Simon have furnished final proof of the actual distinct specificity of the giardias of man and of rats and mice. I have carried on similar studies with giardias from the tadpole, dog, rabbit and guinea pig, and have found certain constant measurable characteristics that prove that the form living in each of these species is of specific rank. Giardias have also been reported from cats, sheep, pigs and birds. Whether these are likewise specific to their hosts remains for further investigation.

Just what the relation is between many entozoic flagellates and their hosts is not well known. In one case, however, we are now able to state definitely that the relationship is one of symbiosis. I refer to the work recently completed by Dr. Cleveland. His investigations were carried on with the intestinal flagellates of the white ants or termites. A study of the various families of termites with respect to their entozoic flagellates revealed the fact that termites that feed on wood are all provided with flagellates. This indicates that some relation exists between the presence of these flagellates and the wood-feed-

ing habit. Grassi claims that heating an infected termite deprives it of some of its intestinal protozoa. Acting on this suggestion, it was found that by incubating termites at 36° C. for 24 hours, *all* the intestinal protozoa could be destroyed without apparent injury to the termite host. These defaunated termites, however, were unable to digest wood and gradually starved to death on what is ordinarily their normal diet. If, however, they were fed on the products of fungus digested cellulose, they continued to live indefinitely without the presence of intestinal flagellates, although still unable to digest wood. Their ability to digest cellulose, which is the principal food material in wood, was regained when they were reinfected with intestinal flagellates. Experiments with a number of species of termites, both those that feed on wood and those that do not, seem to prove conclusively that these flagellates actually digest cellulose within the intestine of the termite and that the termite depends for its nutritive material, and hence for its very existence, upon the products of this digestion. In connection with the necessity of the presence of these flagellates for the continued existence of the termites, it is interesting to note that termites exhibit one of the most perfect methods known of the transmission of entozoic protozoa from one host to another. The young termites are fed by certain members of the colony on so-called proctodeal food. This is material in a partly digested condition that has passed through the intestine of the termite, where it becomes loaded with intestinal flagellates, and is then transferred immediately to the young termites, all of which are thus assured of infection. It thus appears that the termites can not exist without the presence of their intestinal flagellates, and the flagellates of course can not live except within the intestine of the termites; a true case of symbiosis is therefore established.

I have presented to you, I am afraid very inadequately, the results of some of the investigations that we have carried on with entozoic protozoa. We have studied a number of other species of entozoic forms and have also devoted some of our attention to free-living species. Of

the former I may mention the life-history studies of Dr. Hogue on the amoebae living in the oyster and her comparison of these amoebae with tissue culture cells; morphological studies of the human entozoic amoebae, *Iodamoeba williamsi* and *Dientamoeba fragilis* by Drs. Taliaferro and Becker; the cultivation and morphological study of an endamoeba, *E. barreti*, by Dr. Taliaferro, Dr. Barret and Mr. Holmes; the accurate description of cysts of *Endamoeba cobayae*, by Mr. Holmes; my investigation of *Cytamoeba bacterifera* in the red blood cells of the frog; life-history and morphological studies of *Crithidia gertrudis* and experimental studies of the relation between insect flagellates and leishmaniosis by Dr. Becker; observations on nuclear division within the cysts of *Chilomastix mesnili* by myself; on nucleo-cytoplasmic relations in *Opalina larvarum* in conjunction with Dr. Wu, and on nuclear phenomena in a balantidium from the monkey with Mr. Holmes. The free-living protozoa thus far used by us as research material include suctorians studied by Dr. Root, and arcellas by Dr. Reynolds and myself.

To most zoologists the organisms to which I have referred to-night are merely names. Nevertheless, they constitute a large and important section of the animal kingdom and furnish material for investigations of great practical and biological significance. Most significant of all, it seems to me, is the study of the relations of the entozoic species to their hosts, involving not only the great problem of the evolution of parasitism but also problems that require excursions into the field of bacteriology, immunology, chemistry and various other sciences. Scientific knowledge must, in protozoology as elsewhere, precede the successful determination and application of control measures. With this in mind, therefore, we have devoted ourselves particularly, as I have attempted to show this evening, to human entozoic species such as the malarial organism, trypanosomes and intestinal flagellates, etc., and have endeavored by carefully controlled experiments to learn something of their relations to their hosts and how such relations may be employed in the field of preventive medicine.

THE STRUCTURE OF THE VERTEBRATE EYE
AS AN INDEX OF DEVELOPMENTAL DEFICIENCIES: WITH THE BEARING ON
RECENT INHERITANCE STUDIES

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THE embryonic development of vertebrate animals normally takes place at definite rates. The rate changes during different developmental stages, but is in general typical for the given species. Unusual conditions arising in the environment tend primarily to alter or interfere with the usual rate of development. It is probably also true that changes may take place within the germ-cells or within the embryos themselves which primarily tend to give rise to a new developmental rate. The modification in rate may be limited to certain embryonic periods or may exist throughout development. In any case, should the change in rate be sufficiently marked, it is followed by modifications in various structural formations. The structure to be most modified will vary, depending upon the stage of development during which the change in rate occurs. The organ or part which should at the time be developing at comparatively the most rapid rate is most affected by the particular interruption. The development of the body axis, eyes, ears, mouth, gills and certain of the alimentary glands may be modified with varying degrees of success by treating the embryo at the several critical moments determined for the origin of these structures. It can be definitely shown in almost every case that the modifications in structure result from an alteration in the developmental rate.

When an organ is structurally arrested or altered by a change in the rate of development, it rarely recovers from this modification, even though a normal rate of development be subsequently established. The failure to recover is probably due to a competition of some kind,

which seems to exist among the developing organs of an embryo. When a part has lost its opportunity to arise or develop at the proper time it is unable to attain this aim at a later moment, since other parts have come more or less into developmental supremacy. There may simply be a change in metabolism or rate of oxidation occurring in the several organs at various times of development. For a complete consideration of the effects of developmental rate on the origin and structure of organs in the embryo, the reader is referred to a previous paper on the subject, Stockard ('21).

If the above propositions are correct it follows that any organ or part which develops in a comparatively active manner throughout a long period of embryonic life is particularly liable to injury through changes in rate. The vertebrate eye is distinctly such an organ.

PERIOD AND EXTENT OF EYE DEVELOPMENT

Briefly surveying the development of the vertebrate eye, it is found to be the first actual organ to arise in the embryo and appears as an enormous outgrowth from the forward end of the neural folds. The appearance of the neural plate is almost the first visible sign of the establishment of the embryonic line or axis and follows closely after gastrulation. The neural plate scarcely begins to form its lateral folds before the anterior end becomes greatly widened, and the first observable step in the origin of the eye occurs. The two great optic vesicles grow out laterally at a rapid rate and invaginate to form the huge optic cups. No investigator can view these processes as they proceed in the living embryo without being impressed with the comparatively great amount of developmental energy necessary to bring about such growths, which in themselves constitute a large fraction of the entire mass of the embryo.

Another neighboring growth of considerable dimensions begins in the ectoderm overlying the optic vesicle, first a thickening, then an invagination of the thickened

area and finally a large, somewhat spherical, mass is constricted away to differentiate into the crystalline lens of the eye. The primary optic cup, or future retina, and the accessory parts of the eye continue an active growth and development, so that even in late embryonic stages the eye still constitutes a great portion of the body, instead of being the comparatively small organ of the final adult head.

Thus the eye arises soon after the primary embryonic axis is established, and its development continues with the differentiation of the retina, the ingrowth of the optic nerve fibers from the retinal ganglion along the optic stalk and the addition and differentiation of numerous accessory parts, lens, humors, glands, muscles and lids. Nothing in developmental dynamics is more striking than these processes, which finally produce the vertebrate eye. And interruptions during development or any deficiency in general developmental energy are, therefore, quite likely to mar the full perfection of the eye in some or all of its parts. It is well known that the eye frequently shows maldevelopment in both its primary and secondary structures, and this fact is to be correlated with its long period of development.

SUSCEPTIBILITY OF THE EYE DURING DEVELOPMENT

The peculiar susceptibility of the eye to developmental modifications may be illustrated by a consideration of specific cases. It has been shown by the writer and a number of others that when the eggs of marine fish are developed in sea-water to which has been added either salts, sugars, substances with anesthetic properties or various other substances that the embryos resulting present a wide range of defective eye conditions. The development of one or both eyes may be entirely suppressed, or either one or both eyes may show all degrees of microphthalmia and maldevelopment. One eye may be perfect and the other absent, or both may be equally defective. It would seem as though one eye frequently pos-

sessed a developmental advantage over the other and that a condition which completely suppressed the right eye, for example, might have no effect on the left. Further, it appears to be quite possible that when only one eye is handicapped the other eye actually profits by the suppression of its mate, since it so often develops unusually well. There is likely some form of competition between the two eyes.

Exactly similar eye conditions may be produced by subjecting the eggs, during early stages, to low temperatures or by reducing their oxygen supply, in both cases slowing the rate of development. The eye response is, therefore, general and tends to follow any treatment that depresses the development of the embryo.

Leplat, '19, has shown the amphibian embryo to respond in a manner similar to the fish when subjected to unusual chemical environments. The eye is frequently abnormal and of the same general types as those cited above.

When the bird's egg is exposed to the action of volatile substances, such as ether or alcohol, I have found, as Féré had previously shown, that enough of these fumes penetrate the shell to affect the developing embryo. These embryos also frequently develop abnormal eyes of exactly the same type as those in the fish. It is thus evident that the eye is a susceptible organ in all classes of vertebrates and may be easily modified by changing the environment of the developing egg.

The mammalian embryo presents a somewhat different case on account of its internal development and, therefore, better protection. But it is found that the female guinea pig may be so treated with ether or alcohol as to affect the developing embryos and cause them to form abnormal eyes. In the case of the female mammal the treatment used may act directly upon the developing embryo if taken into the placental circulation, and the conditions, therefore, become comparable to those acting upon the externally developing eggs of fish, amphibians and birds. Guyer and Smith ('18) have obtained similar re-

sults by treating the pregnant rabbit with fowl serum containing lens antigen.

The problem may, however, be taken a step further by treating the male mammal. We have found that after treatments of sufficient duration and intensity the male guinea pig may sire by normal females young with various defective conditions of their eyes. These animals may be entirely eyeless, one-eyed or with different degrees of microphthalmia, various opacities and abnormalities of the lens, and other types of maldevelopment. The maldevelopment in this case is not due to unusual conditions in the environment surrounding the embryo, since the mother is untreated and is a tested normal animal.

The eye abnormalities of the young sired by a treated male must be attributed to an injury or modification in the germ-cells. The injury has affected the spermatozoon in such a manner that when it fertilizes a normal egg the developmental capacity of the zygote is lowered and a defective individual is produced. It seems highly probable that the chromatin in the germ-cells has been affected and the subsequent generations arising from this injured germ-plasm continues to be inferior as compared with the control generations. The treatments have brought about a general lowering of the developmental capacity of these germ-cells and they give rise to generations of defective and under-developed individuals. No specific response has been detected. The effect is general, and the young animals frequently show defects of the eyes closely similar to those induced by slowing the developmental rate of other vertebrate species. One might imagine that the treatment had acted to disturb the genes which determine the normal coordination of the developmental processes. There may be certain genes that regulate the rate or activity of development, just as there probably are genes determining the limits of growth. These physiological factors seem disturbed, and the results are general rather than specific for definite structural characters. It might be that groups of genes are affected in a way to disturb the usual develop-

mental processes. The effects are more far-reaching than if a single gene was involved, unless there be only one gene which definitely determines the rate of development.

The treatments affect the germ-cells of the mammal and cause them to give rise to subnormal offspring, and the defective conditions, when once induced, are transmitted in the race until the defective group finally succumbs.

With the treatments we have used on the guinea pig, only part of the germ population would seem to be injured, while the more resistant germ-cells apparently escape, but the details of these experiments are not of importance in the present considerations.

IS THERE SPECIFIC INHERITANCE OF VERTEBRATE EYE ANOMALIES?

From the above cited studies of the behavior of the eye during development and the transmission of eye defects in degenerate stocks, we are inclined to believe that any treatment of a mammal which would tend to injure its germ-cells might very probably cause maldevelopment of the eye. Several workers have recently recorded important results of this kind which we may briefly examine.

Guyer and Smith ('18, '20) have carried out an ingenious series of experiments with injections of lens antigen into rabbits. Their method was to crush the crystalline lenses of rabbits and inject this material into chickens and later to collect the blood from the injected chicken. The serum which contains an antigen against the rabbit lens is then administered in definite doses to the rabbit. The doses used are slightly less than a fatal amount; when a little more than the experimental dose is given the rabbit dies. The treatment is very toxic. The antigen does not injure the lenses of the rabbit into which it is injected, but it affects the germ cells of this rabbit and some of its offspring may show defective eyes and cloudy or badly formed lenses. These eye defects are transmitted to later generations and seem to be inherited in

this line of rabbits. Guyer and Smith have, therefore, interpreted the result as a specific response of the germ-plasm to the lens antigen and hence the defective lenses of the progeny. The facts are extremely interesting, but do they not lend themselves to a different and more probable explanation?

In the first place, the control animals are treated with normal fowl serum and with so-called antigen of rabbit testis which may be somewhat less toxic for the rabbits than is the lens antigen. These control treatments may be only passive substances or their action may not be of the type to affect the germ-cells at all; certainly two different treatments rarely act in a similar way on the same organ.

In the second place, if the lens treatment has a specific action on the germ-cells, why is not the lens of the offspring alone affected? Guyer's specimens show numerous defective conditions of not alone the lens but of the entire eye ball. The eye may be almost absent, as in the alcoholic guinea pig. Or suppose the treatment was specific for not only the lens but for the entire eye—then why do so many of the progeny show defects in only one eye, while the other eye is perfect? It seems somewhat peculiar to inherit a specific eye condition in one eye and not in the other. Yet it must be recalled, as Guyer points out, that certain conditions such as polydactyly, which is definitely inherited, is frequently asymmetrical in its expression.

These eye conditions are, however, exactly what would often obtain from a general injury of the germ-cells, causing a lowered developmental capacity, and just such a condition is transmitted in our defective guinea pig stocks. The eye reactions recorded by Guyer and Smith seem to be much like typical developmental arrests and may not be specific reactions to a definite treatment. They may be genetic in the sense that certain genes are affected by the treatment and are so disturbed as to give improper developmental coordination. The germ is modified so as to be incapable of well-organized develop-

ment and this altered condition of the genes may be transmitted, but the expression which indicates the new condition is general and secondary and is merely the result of a lowered developmental capacity or an arrest.

It is well known that similar eye defects—monophthalmia and asymmetrical microphthalmia—often occur spontaneously in weak or degenerate races of birds and mammals. Such conditions may appear for several generations, and are indicative of general maldevelopment in the stock. But that there is a specific genetic basis for eye defects may be seriously questioned when one considers a type of developmental derangement which I have recently studied, the commonly known double or twin conditions. In double-headed embryos and in united twin specimens the components frequently differ in size and, when they do, the smaller one is invariably deformed. The smaller component has a slower and more irregular developmental rate than the larger, and it shows every kind and degree of eye abnormality that is mentioned above. The lens is frequently abnormal and all grades of microphthalmia occur in either one or both eyes. This type of specimen conclusively demonstrates that the eye defects are the result of a depressed development, since they only occur in the smaller, poorly formed, weak component and not in the larger component which is as typically normal in its structure as a single individual might be. Certainly no defect in the smaller component could be genetic in origin, since the two components are derived from a single fertilized egg and the genetic composition of the smaller component is the same as that of the larger. The double specimen with unequal components serves to illustrate in a most convincing manner the extreme susceptibility of the vertebrate eye to any developmental disadvantage and shows it to be the most reliable and constant indicator of developmental deficiencies.

No other organ or part in the lesser component of the double specimen is so constantly deformed as is the eye. In view of these facts it would seem highly probable that a transmitted defect of the eye is not a specific eye condi-

tion due to a changed eye factor but is far more likely due to a modification or change in those factors in the germ which have to do with a proper developmental coordination and capacity.

Very recently Bagg and Little have reported that after mice are treated with a definite dosage of X-ray a germinal modification is produced which gives rise to maldevelopment of the eye along with several other structural deformities. The specimens showing the abnormal eyes may be selected out and finally bred so as to give approximately 100 per cent. of individuals with abnormal eyes. The condition is inherited in the race as a Mendelian recessive. The effects of the X-ray treatment seem to be much more universal on the germ-cells than either our alcohol treatments or Guyer and Smith's lens antigen, and for this reason Mendelian expectations are more nearly attained. That is, it becomes possible to obtain a purely breeding recessive. In our alcohol experiments it seems very probable that with the treatments used only a fraction of the entire germ-cell population is modified and a certain lot escape. We are thus breeding animals containing a mixture of normal and modified germ-cells and the number of defective animals in each generation is below the Mendelian expectation. Guyer's results seem somewhat the same. Bagg and Little have, however, succeeded in getting with X-ray a modification of the total germ population and their genetic records are far better than ours.

The question still arises regarding the specificity of the eye defects Little and Bagg find. These eye conditions are all of exactly the same nature as those described above as resulting from developmental interruptions.

It is again difficult to think of the eye defects in X-ray mice as being due to a mutation or change in a particular gene determining eye structure. We can certainly not think of the similar eye conditions as having been due to any such specific gene alterations in the directly modified embryos of fish, birds and rabbit. Here again it may be imagined that the X-ray treatment has so modified the

chromatin as to lower its capacity for giving rise to a zygote with a well-regulated normal development. Masses of the chromatin or blocks of genes may be disturbed in some way, and such modified masses may be segregated in a Mendelian fashion. The fact that the F_1 individuals do not show the defects would not argue against such a gross modification of the chromatin, since a non-disjunction or some form of melting together of the genes might occur in a gradual manner and might not be brought about at once in the directly treated germ-cells of the exposed P_1 generation.

It is difficult at present to speculate on an exact mechanism by which these defective conditions are transmitted. And it may be possible that some simple mass action of altered chromatin may be involved. It is true in both Guyer and Smith's rabbits and Little and Bagg's mice that the number of defective individuals increases through several generations before high proportion of such individuals are reached. This might indicate that the eye condition was dependent upon several factors instead of one or only two. But it might also indicate that when the defective lines are closer inbred a larger proportion of modified chromatin is present in each zygote and, therefore, the development is more uniformly sub-normal as is the case among a degenerate family of animals.

BLIND FORMS IN NATURE

There is a condition widely present in nature which is closely related to the problem under discussion. I refer to the blind and eyeless forms of fish, amphibians and a few reptiles which live in a dark or cave environment. Numerous speculations have been advanced as to the origin or cause of these blind forms, and it may not be altogether harmful to add another, particularly since the foregoing consideration of the susceptibility of the developing eye tends to substantiate the view to be presented.

All the blind vertebrates living in dark and protected places have close relatives with perfectly developed eyes

living in the light. The blind form in every case, I believe, is a weaker, less well-developed animal than its nearest relative. The more hardy and active form is never the cave-living one. It might be suggested that the blind animal is less active because of its blindness, but if the blindness be due to poor development then a low activity would be correlated with it.

The embryos of all blind vertebrates that have been studied show that the optic vesicles, and often lenses, are formed and then become arrested, and either completely degenerate or persist as maldeveloped eyes buried in the head. Those forms that merely burrow and hide temporarily under cover, such as *Rhineura*, the Florida burrowing lizard, exhibit the greatest variation in the degree of degeneration of the eyes. Eigenmann noted that the lens was absent from 50 per cent. of the eyes of *Rhineura* and was variable when present. Such animals are not forced to seek a permanent abode in the dark. Other forms that live constantly in the dark of deep caves show a more uniformly degenerate eye with little variation in degree of development.

These blind animals may not have inherited a specific eye character, but may rather have inherited a more or less definite change in their developmental rate or capacity during a period peculiarly critical for the developmental expression of the eyes. A single mutation may have been responsible. The darkness has not on any substantial ground been shown to be a causal factor in such conditions. If these animals represent a genetic type of subnormal development then it is rather logical to presume that they will timidly seek cover and come to reside in dark places, while the bolder and more fully developed near relative would have no such tendency to seek the shelter of caves.

CONCLUSION

Finally, then, there is remarkable uniformity of type and condition in the maldevelopment of eyes, from direct injury of the egg or embryo, in the offspring of mam-

mals with modified germ-cells as a result of various treatments, and under natural environments. The eye is definitely a susceptible and responsive organ during development. In view of this it seems a safe attitude to consider the vertebrate eye as an indicator of developmental deficiency and to question the specificity of origin of any eye anomaly. It may further be appreciated that when the eyes are structurally deformed or deficient in an individual there is a likelihood that other organs and parts have also suffered from the same arresting cause. This is well shown in many cases by the experimented embryos, our alcohol treated guinea pigs and the X-ray mice of Bagg and Little. Conversely, it is probably more often true that when developmental arrests are present in various body organs, the eyes of such an individual are also structurally deficient.

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A SEXUAL ACTIVITY RHYTHM IN THE FEMALE RAT¹

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INTEREST in periodic activity led us to the discovery of a regular four-day rhythm in the variation of the daily amount of activity in the female rat. Four experiments were performed with a total number of nineteen rats—fourteen females and five males. In these experiments, each rat was placed in an activity cage, which consisted of a small living cage and a revolving drum. The revolving drum was attached to the living cage in such a way that the animal in the activity cage had free and easy access to the revolving drum (13 inches in diameter.) A ratchet cyclometer was connected to the revolving drum by a system of levers and registered automatically the revolutions of the drum in both clockwise and counter-clockwise ways. Thus, the daily records of the readings of the cyclometers served as a fairly accurate measurement of the daily variations of the amount of activity. Environmental conditions were very carefully controlled through all the experiments; the animals were kept under constant illumination, in fairly uniform temperature (around 20° C.), and with food and water all the time. The fourteen females and five males experimented on all gave the same results. Only females which had reached sex-maturity showed the regular four-day rhythm, and no male showed any such regular rhythmic variations.

In Fig. 1, a typical activity curve of a female rat is given. The number of the revolutions of the revolving

¹ The full account of this work is going to be published as No. 6 of the Comparative Psychology Monographs.

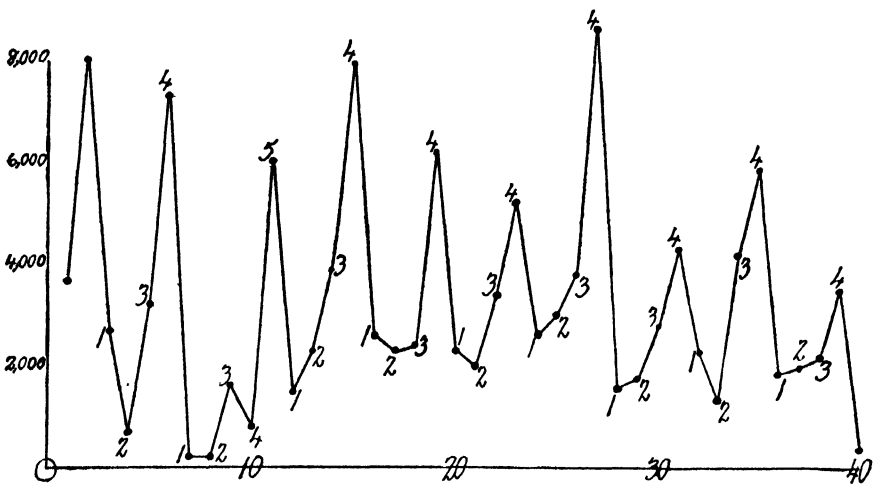


FIG. 1. A typical activity curve of a female rat. Rat D2.
Age: 108-148 days.

drum is plotted on the ordinates and the number of days of the experiment on the abscissae. This curve shows many "peaks." If the intervals between these "peaks" are examined, it will be found that nearly all of them are four days in length (with only one exception). The drop in activity after each "peak" is generally very large. It is usually about 60 per cent. and sometimes amounts to more than 90 per cent.

On all the fourteen female rats experimented on, a total of 192 intervals was observed. The mode (98 instances) of the distribution curve of these 192 instances is four days, and the range of variation from two to eleven days.

Attempt was then made to find out whether this regular activity rhythm is related to the oestrous cycle. According to Long and Evans (1), the oestrous cycle in the rat is on the average four days in length, varying from three to thirteen days. The oestrous cycle is divided into five stages, which can be differentiated in a living animal by microscopic examination of smears of vaginal content and by the sexual behavior of the animal. The results of Long and Evans on these points are given in the following table:

Stage	Length in Hours		Cellular Elements of Vaginal Smear	Sexual Behavior
	Mode	Average		
1	12	14.2	Nucleated epithelial cells.	Not in "heat" ²
2 (period of oestrus)	27	38	Cornified epithelial cells.	In "heat"
3 (period of ovulation)			Cornified epithelial cells.	Not in "heat"
4	6	7.8	Cornified epithelial cells and leucocytes.	Not in "heat"
5	48	53	Nucleated epithelial cells and leucocytes.	Not in "heat"

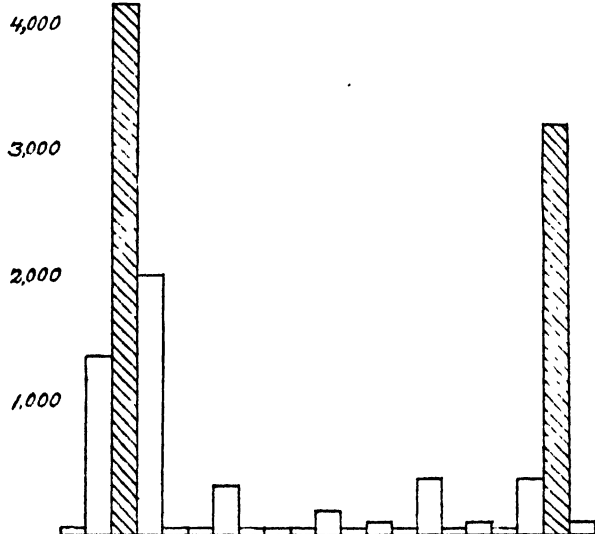
² Some animals may go into "heat" in the latter part of this stage.

On the basis of these results, experiments were carried out to find out the time relation between the activity rhythm and the oestrous cycle. It was found that, in fifty cases, the vaginal smear made on the day of the "peak" of activity nearly always showed cornified epithelial cells alone (with only four exceptions, in which cases the smear made on the day before the "peak" showed cornified epithelial cells.) It was also found that the female rats were in "heat" on the day of the "peak" of activity. These findings showed that the activity rhythm bore a definite time relation to the oestrous cycle and that most likely a female rat was active during the period of oestrus. Another experiment with four animals was then performed to determine the exact time relation between the two periodic phenomena. In this experiment, readings of the cyclometers were taken and vaginal smears made every six hours (Noon, 6 P. M., 6 A. M., and Midnight). Each animal was taken out of its cage at these times and offered to a male in an ordinary rat cage. The sexual behavior of the female was closely watched for five minutes, actual copulation being prevented. At the end of this period of observation, the female was returned to its activity cage. The results of

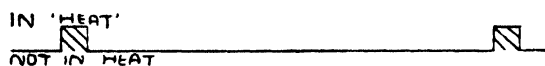
this experiment demonstrated that a female rat is most active during stage two of the oestrous cycle, when vaginal smear shows the presence of cornified epithelial cells alone, and the animal is receptive (see Fig. 2).

ACTIVITY

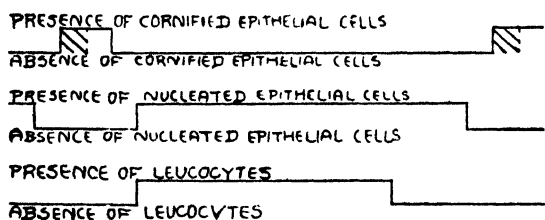
NO. OF REVOLUTIONS OF
THE REVOLVING DRUM



SEXUAL BEHAVIOR



FINDINGS OF SMEAR EXAMINATIONS



TIME IN SIX HOUR PERIODS

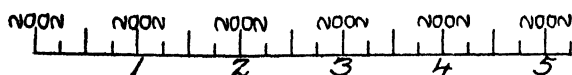


FIG. 2. Graph showing the exact time relation between the activity rhythm and the oestrous cycle. Rat P1. Age: 130-135 days.

to note that there was a big and sudden drop in activity after conception. The activity level was continuously low throughout both periods of pregnancy and nursing. Daily activity records were also taken for a sufficiently long period of time after weaning to see that the activity rhythm returned, and the activity came back to its normal level. It was found that it took more than five days for the return of the normal level of activity and more than ten days for the return of the activity rhythm (Fig. 3).

A second experiment was carried out with four just weaning females (20 days old) to ascertain whether the activity rhythm was present before sex-maturity. The results of this experiment were: (1) that the activity was on a very low level and without any indication of rhythmic variations when the females were 20-40 days old; (2) that after this there was a period (about 10-20 days) of slight increase in activity and with somewhat irregular rhythm; and (3) that the high "peaks" and regular cycle suddenly appeared. Some experiments made later on showed that oestrous cycle as determined by smear examination appeared in the second period but it was of irregular length, and that high "peaks" and regular activity rhythm came into existence at the same time with the appearance of regular oestrous cycles.

Another experiment deals with the effect of ovariectomy and hysterectomy. The results came out just as expected, that is, activity rhythm ceases with double ovariectomy, but persists with the removal of the two horns and body of uterus. It is also very interesting to note that the ovariectomized females have as low levels of activity as the immature ones.

The main results of the experiments briefly described above are: (1) Adult female rats show regular four-day rhythm in activity; (2) The activity rhythm bears a definite time relation to the oestrous cycle, the animal being most active during oestrus and before the onset of ovulation; (3) The activity rhythm is absent in cases when

the oestrous cycle is not present, for instance, during pregnancy and lactation, before puberty and after ovariectomy.

Then, the conclusion is that this activity rhythm in the female rat is a sex rhythm and depends on the regular periodic functioning of the ovaries.

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INBREEDING THE RHODE ISLAND RED FOWL WITH SPECIAL REFERENCE TO WINTER EGG PRODUCTION (PRELIMINARY REPORT)¹

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DIVERSE opinions exist concerning the advisability of inbreeding flocks of chickens. The opinion is rather general among poultrymen that low vigor, low hatchability and high mortality, as well as many other faults, may result from close matings. The fact is very well demonstrated that the uniformity that comes from inbreeding is the result of a nearer approach to homozygosity in Mendelian factors. With this greater homozygosity in desirable characters comes the homozygosity for undesirable characters. If the assumption is true that desirable characters—those that have made the perpetuation of the race possible—are dominant, we have a means of explaining why the more heterozygous (those not inbred) are the more vigorous. This may be a means of explaining why flocks deteriorate in some desirable qualities if bred without the introduction of new blood. If the breeder were able to start with a foundation homozygous for these so-called desirable characters, it is not inconceivable that inbreeding could go on indefinitely without any defects occurring.

The question arises: Are the standards for selecting breeding fowls correct to the extent that their application enables the breeder to distinguish the kind of individuals he really wants? May it not be possible that homozygous individuals would not meet the requirements in individuality? Here enter the effects of heterosis. Because of the great complexity of modern breeds of poultry, ideals

¹ Contribution No. 1 from the Massachusetts Agricultural Experiment Station.

may call for a type exhibited only by heterozygous individuals, and when attempt is made to approach homozygosity by inbreeding, the progeny may be lacking in what is called good vigor, high hatchability, etc.

All poultry breeders are striving to establish uniformity in their flocks. Experience teaches that the only known method of securing uniformity is through inbreeding. But while inbreeding for uniformity in color, type, weight, etc., the breeder may be confronted by low vigor, low fertility, high mortality, etc. Such results would at once tend to condemn close breeding. The fact remains that close breeding has been used for one purpose, *viz.*, to secure uniformity. But the uniform individuals do not possess the vigor sought for. This vigor may be had as Wright (1922) has shown in guinea pigs by mating to unrelated stock, but how can uniformity be retained if unrelated stocks are brought together? The method is simple and is the one outlined by East and Jones (1919) for breeding corn: Develop several unrelated inbred strains to a high degree of uniformity for desirable characters of the breed, then cross these and the uniformity remains and renewed vigor results from heterosis.

A preliminary experiment in inbreeding Rhode Island Red chickens was undertaken by Dr. H. D. Goodale at the Massachusetts Agricultural Experiment Station in the spring of 1919. Four crops of chicks have been secured to date. This preliminary report covers the results on birds hatched in 1919, 1920 and 1921 all of which were given an opportunity to demonstrate their winter egg-laying ability.

FOUNDATION STOCK

The birds used in this experiment were all pedigree Rhode Island Reds that had been bred for egg production for six years at this station. One hen No. B357 was mated to her son B3808 in 1919. This hen made a second year record of 172 eggs. She was used for breeding for four seasons and lived to be over five years old. There was no common ancestry in the pedigree of this hen for six

generations, and she was not related to her mate, the sire of B3808. Hen B357 was fully up to breed requirements in size; her pullet year record was 286 eggs, and 73 per cent. of her eggs were fertile the first year with a hatchability of 63 per cent. Males in all cases were selected carefully to secure the best individual where several were available. All daughters were trapnested throughout the winter unless physically unfit. Other hens used during the first year were two full sisters to B357, one distantly related hen and four unrelated hens. All these hens were yearlings and each had a trapnest record.

GENERAL PLAN OF MATINGS

The general plan was to concentrate the blood of B357. A son of hers by a son was used in 1920. In 1921 two sons by a son of this hen were used. Unrelated hens were used as checks the first year. Female descendants that were full sisters were mated to their full brothers in 1921. The combined results are presented in Table I.

Table I gives results of mating male B3808 to his dam, two of her full sisters and one more distantly related hen in the inbred group. In the check group are four females all unrelated to B3808. A reasonable expectation would be for a considerable degree of uniformity in winter egg production in the daughters of the first three hens because they are full sisters and are mated to a son of one of them. This point will be considered later.

A general tendency for the mean winter egg production of the daughters to fall below their dams is observed. This is to be anticipated for no other reason than that the dams are a selected group and far excel the rest of their full sisters in winter egg yield. In those few cases where the average of the daughters excels the dams, the explanation lies in the effects of factors for high production contributed by the male.

The possible relation between the degree of inbreeding and mean winter egg production is interesting. The 25 inbred daughters of B3808 averaged 56.4 ± 17.50 winter

TABLE I
PRELIMINARY INBREEDING EXPERIMENTAL RESULTS
1919 Hatch

Male Number	Female Number	Winter Egg Record	Winter Rate	Winter Pause Days	Inbreeding of Daughters	Daughters' coefficient of variation for winter egg Production
B3808	B 357	Dam 112 2 Daughters 50.5	Dam 73% Daughters 67%	Dam 0 Daughters 27	25 % Inbred 62.5 % Homozy.	10.89%
B3808	B 359	Dam 106 9 Daughters 60	Dam 80% Daughters 47%	Dam 39 Daughters 41	25 % Inbred 62.5 % Homozy.	48.67%
B3808	B 754	Dam 73 8 Daughters 59	Dam 66% Daughters 52%	Dam 8 Daughters 31	25 % Inbred 62.5 % Homozy.	42.86%
B3808	B 699	Dam 80 6 Daughters 42	Dam 64% Daughters 49%	Dam 0 Daughters 28	6.25 % Inbred 53.12 % Homozy.	36.80%
B3808	B 710	Dam 93 7 Daughters 60	Dam 60% Daughters 65%	Dam 21 Daughters 21	Not Inbred	43.18%
B3808	B 785	Dam 84 7 Daughters 87	Dam 80% Daughters 71%	Dam 4 Daughters 9	Not Inbred	27.00%
B3808	B1357	Dam 72 13 Daughters 58	Dam 67% Daughters 55%	Dam 0 Daughters 20	Not Inbred	32.68%
B3808	B2219	Dam 39 7 Daughters 50	Dam 65% Daughters 62%	Dam 0 Daughters 16	Not Inbred	36.98%
1920 Hatch						
B5806	B 357	Dam 112 5 Daughters 44	Dam 73% Daughters 47%	Dam 0 Daughters 9	37.5 % Inbred 68.75 % Homozy.	46.93%

TABLE I (Continued)

Male Number	Female Number	Winter Egg Record	Winter Rate	Winter Pause Days	Inbreeding of Daughters	Daughters' coefficient of variation for winter egg Production
B5506	B3512	Dam 38 1 Daughter 18	Dam 47% Daughters 18%	Dam 28 Daughters 67	25.00 % 62.5 % Inbred Homozy.	
1921 Hatch						
B8273	B8318	Dam 76 4 Daughters 50	Dam 70% Daughters 53%	Dam 0 Daughters 14	28.125% 64.062% Inbred Homozy.	20.00%
B8273	B9796	Dam 58 1 Daughter 44	Dam 81% Daughters 44%	Dam 0 Daughters 31	28.125% 64.062% Inbred Homozy.	
B8273	C 23	Dam 18 4 Daughters 28	Dam 41% Daughters 31%	Dam 19 Daughters 53	28.125% 64.062% Inbred Homozy.	29.11%
B8574	B8318	Dam 76 3 Daughters 13	Dam 70% Daughters 43%	Dam 0 Daughters 8	28.125% 64.062% Inbred Homozy.	23.91%
B8574	B9651	Dam 26 2 Daughters 51	Dam 54% Daughters 62%	Dam 0 Daughters 0	28.125% 64.062% Inbred Homozy.	20.00%
B8574	B9796	Dam 58 1 Daughter 55	Dam 81% Daughters 72%	Dam 0 Daughters 0	28.125% 64.062% Inbred Homozy.	
B9275	B 357	Dam 112 2 Daughters 57	Dam 73% Daughters 72%	Dam 0 Daughters 4	43.75 % 71.87 % Inbred Homozy.	9.45%

eggs; the 34 outbred daughters averaged 63.24 ± 15.675 eggs. There is, therefore, no significant difference in the mean winter egg production of the daughters of this male that are inbred from those that are outbred. Because of the large number of Mendelian factors concerned, as well as a large number of environmental factors, significant differences could only be expected if several hundred daughters of a given male are considered. The fact that no significant difference in winter egg yield comes from mating the same male to related females and unrelated females indicates that both groups of females were of about the same factor constitution for egg production.

Unfortunately, we are unable to check the inbred pullets hatched in 1920 and 1921 against outbred daughters of the same sire, because the males used in inbreeding were not mated to any unrelated hens. One obvious fact indicated by the table is the decline in winter egg production of both dams and daughters used during these two years. The fact is evident that the descendants inbred to hen B357 did not lay as many eggs as B357.

Winter rate of production is calculated by dividing the number of eggs laid during the pullet year before March by the number of days between the first and last egg. One very noticeable feature is the apparent decline in rate as inbreeding progressed. The outbred birds hatched in 1919 exhibit no significantly higher rate than their inbred sisters. During the second and third year of the experiment, however, the winter rate became so low that the mean egg production is very mediocre.

The length of winter pause did not increase with the degree of inbreeding and behaves in ordinary Mendelian fashion, as will be shown later. In general, the decreased winter egg production with the increased degree of inbreeding may be explained as due largely to decreased early maturity and decreased winter rate of production.

The percentage of inbreeding of the daughters is calculated by Wright's (1922) method and the theoretical degree of homozygosity is calculated for each inbred mating. The latter figures serve as an indicator of the still

large percentage of heterozygosity in rather closely inbred birds. Inbreeding has here operated to make homozygous some of the factors detrimental to high egg yield. Evidently the foundation hen, although a good individual as far as individual criteria may be recognized, carried factors which when intensified reduced the winter egg production as the experiment progressed.

The gross effects of inbreeding upon winter egg production may be studied through the daughters' coefficients of variability as given in the last column of Table I. The 1919 hatch shows a rather wide coefficient of variability as might be expected. The smaller range in variability of outbred daughters compared with inbred daughters is contrary to expectation and evidently due to experimental error entirely. The very large coefficients of variability that exist when full sisters are considered has caused many to question the inheritance of egg production according to Mendelian laws. Coefficients of variability are consistently smaller for the 1921 daughters, even though the number from each dam is small and more males were used than in 1919, so that the results are subject to larger error. The general assumption may be drawn from these very limited results that variability in winter egg yield may be reduced by breeding methods.

EFFECT OF INBREEDING ON WEIGHT AT FIRST EGG

Body weight is often considered an index of vigor. In Table II are presented the weights of each dam at her first egg, together with the average weight of all her daughters at first egg.

Table II clearly shows that the weight at first egg was amply maintained regardless of inbreeding. The second and third year inbreds were older when the weight at first egg was recorded and this would give them an advantage.

FERTILITY AND HATCHABILITY

Fertility and hatchability records are available on only a limited number of the hens included in this report.

TABLE II
WEIGHT OF PULLETS AT FIRST EGG

1919 Hatch	Lbs.	Oz.	1920 Hatch	Lbs.	Oz.
B357	4	10	B357	4	10
2 Daughters	5	8	5 Daughters	5	0
B359	5	9	B3512	6	8
9 Daughters	5	15	1 Daughter	6	7
B754	7	0	1921 Hatch		
8 Daughters	5	14	B8318	5	2
B699	5	6	8 Daughters	5	1
6 Daughters	5	8	B9796	5	5
B710	5	0	2 Daughters	5	2
7 Daughters	5	2	C23	4	11
B785	5	4	4 Daughters	5	9
7 Daughters	5	1	B9651	4	8
B1357	4	10	2 Daughters	4	11
13 Daughters	4	13	B357	4	10
B2219	6	3	2 Daughters	4	6
6 Daughters	5	13			

Records are available for all hens used as breeders, but for few of the daughters of each hen. A very noticeable decline has been observed particularly in hatchability and to lesser extent in fertility as the inbreeding increased. Foundation hen B357 has a record as follows for her four breeding seasons:

HEN B357 HATCHED 1917

1918 mated to B115		1920 mated to B5806	
Per cent. fertile	73	Per cent. fertile	100
Per cent. fertile hatched	63	Per cent. fertile hatched	50
1919 mated to B3808		1921 mated to B9275	
Per cent. fertile	99	Per cent. fertile	96
Per cent. fertile hatched	49	Per cent. fertile hatched	67

This hen shows a rather low degree of hatchability coupled with a high degree of fertility. With such a foundation, low hatchability might be expected.

THE GENETIC BASIS OF WINTER EGG PRODUCTION

Goodale and Sanborn (1922) have already pointed out that the egg record of the Rhode Island Red hens in the Massachusetts station flock depends upon five main characteristics: maturity, rate, broodiness, persistency and winter pause. In the interpretation of the inbreeding results we have not included persistency and for winter pause we choose to use the term "partial molt" for the

reason that all pauses of more than a week's duration are accompanied by partial molt.

EARLY MATURITY

Early maturity has been pointed out by Goodale and Sanborn (1922) in Rhode Island Reds and by Hurst (1921) in Leghorns and Wyandottes as having an important bearing on egg production. Further studies made by the writer at this station show a negative correlation between age at first egg and annual production during the pullet year of about 43 per cent. with a small probable error. In other words, early maturity is associated with heavy egg yields. Just what the relationship of early maturity is to rate and broodiness has not yet been worked out. Evidence seems to point to an association between extreme early maturity and the occurrence of the partial winter molt, but there is no conclusive evidence on this point.

Results tabulated in this report as well as many more extensive records on the Massachusetts station Rhode Island Red flock seem to indicate that two dominant factors for early maturity are concerned. We call these E and E'. Either combined or alone, simplex or duplex, these factors produce a bird that normally begins laying at 215 days or less. Those birds that lack both factors E and E' (ee' birds) do not begin laying until 216 days or older. The factor E is sex-linked and factor E' is not sex-linked and is independent. One factor is known to be sex-linked because early maturing hens sometimes have entire families of late daughters.

The age at which a pullet lays her first egg, without doubt, depends on a large number of environmental factors. Such factors operate to make different pullets of the same genetic composition for early maturity begin laying at from 150 to 215 days and those lacking both of these factors at from 216 to 300 days or more. A cumulative effect of the factors E and E' has not been demonstrated, but may exist.

The results of the first year of inbreeding are summarized in Table III. Inbred pullets hatched during 1920 and 1921 appear to be abnormal in early maturity and are omitted. These birds seem to be depleted in what might be called "sex vigor." In other words, they fail to begin laying until two or three months older than normal. The general behavior may be illustrated by the age at maturity of the daughters of hen B357 on four successive years. Here the degree of inbreeding advances from 0 in the first year to 43.75 per cent. in the fourth year.

RELATION OF DEGREE OF INBREEDING TO AGE AT FIRST EGG

<i>Year</i>	<i>No.</i>	<i>Daughters Average Age</i>
1918	5	179 days
1919	2	199 "
1920	6	235 "
1921	2	189 "

In Table III are the individual hens mated in 1919 to male B3808 with the genetic formula of each for factors governing early maturity. Opposite each hen is her daughters' classification, together with the probable error of ratio. In only two cases, namely, the daughters of B785 and B1357, is the actual deviation of the ratio greater than the probable error of the expected ratio calculated according to Weldon (1902). Probably hen B785 was an early maturing hen but may have been held back by environment. In the case of hen B1357, only 13 daughters went through the winter normally and are included in this report. Her other nine daughters were abnormal and are not included because they never laid or laid only a few eggs in the fall or developed ovarian disorders and became "nesters." Some of these daughters were late maturing, so that the formula $Eo F'e'$ is thought to be correct.

The agreement of results with theory is close and is mathematically significant. The evidence of two dominant factors for early maturity seems rather conclusive even though the number of birds concerned in this report is small.

TABLE III
EARLY MATURITY
1919 Hatch

Up to 215 days = early
216 days or more = late

Dam	Genetic Formula	Sire B3808 (Ee E'e')	
		Actual	Expected
B 357	Eo E'e'	2	1.75 \pm .31 early
		0	.25 late
B 359	Eo E'e'	8	7.875 \pm .67 early
		1	1.125 late
B 754	eo e'e'	6	6.00 \pm .82 early
		2	2.00 late
B 699	Eo E'E'	5	5.00 early
		0	0 late
B 710	Eo E'e'	6	6.125 \pm .59 early
		1	.875 late
B 785	eo e'e'	7	5.25 \pm .77 early
		0	1.75 late
B1357	Eo E'e'	13	11.375 \pm .80 early
		0	1.625 late
B2219	eo e'e'	5	5.25 \pm .77 early
		2	1.75 late

WINTER MOLT

The winter molt as referred to in this report probably corresponds with winter pauses of considerable length referred to by others. With the Rhode Island Reds there appears to be a close association between pauses of one week or more by pullets in the fall or winter and a partial molt. This fact has been checked up on large numbers. Furthermore, this tendency appears to be inherited on a single factor basis. By using only non-molt breeding females this winter loss of eggs has been greatly reduced. A study of its behavior in male breeders has not yet been made. There may also be linkage between factors for early maturity and molt. The factor M is used here to represent the gene for winter molt.

In the unimproved state, the hen generally begins laying in March or April and continues more or less irregularly for about three months. She then prepares herself for complete molt. By methods of breeding, selecting for extreme early maturity, housing, etc., most of the pullets now begin laying in the fall. If they still exhibit the natural tendency to molt in from two to three months after beginning to lay, they are spoken of as showing winter

molt. Severe weather conditions of winter seem in a measure to check this loss of feathers, so that only a partial molt is observed. The general belief is that molt is associated with a depleted metabolic condition and this may be brought about by the heavy strain of egg laying. Pullets that show no winter molt even though they begin to lay early, evidently differ genetically from the original type in regard to molt.

TABLE IV
WINTER MOLT
1919 Hatch

Dam	Genetic Formula	Sire B3808 (Mm)		
		Daughters		
		Actual	Expected	
B 357	mm	1	1	molt
		1	1	non-molt
B 359	Mm	7	6.75 \pm .88	molt
		2	2.25	non-molt
B 754	Mm	6	6 \pm .82	molt
		2	2	non-molt
B 699	mm	4	2.5 \pm .76	molt
		1	2.5	non-molt
B 710	Mm	5	5.25 \pm .77	molt
		2	1.75	non-molt
B 785	mm	3	3.5 \pm .89	molt
		4	3.5	non-molt
B1357	mm	7	6.5 \pm 1.21	molt
		6	6.5	non-molt
B2219	mm	4	3.5 \pm .89	molt
		3	3.5	non-molt

1920 Hatch

Sire B5806 (Mm)				
B 357	mm	2	2.5 \pm .76	molt
		3	2.5	non-molt
B3512	Mm	1	.75	molt
		0	.25	non-molt

1921 Hatch

Male B8273 (Mm)				
B8318	mm	2	2 \pm .67	molt
		2	2	non-molt
B9796	mm	1	.5	molt
		0	.5	non-molt
C23	Mm	4	3 \pm .58	molt
		0	1	non-molt
Male B8574 (Mm)				
B8318	mm	1	1	molt
		1	1	non-molt
B9651	mm	0	1	molt
		2	1	non-molt
B9796	mm	0	.5	molt
		1	.5	non-molt
Male B9275 (mm)				
B 357	mm	0	0	molt
		2	2	non-molt

There is a very close agreement between theory and result in Table IV. There is not a single instance in a family of sufficient size to calculate the probable error of expected ratio with any degree of accuracy, where the results deviate beyond the limits of probable error. There seems little ground for questioning the heritability of winter pauses of a week or more in duration.

BROODINESS

Hens that go broody spend considerable time not laying during such periods. Broody periods may vary in length from a few days to several weeks. Goodale (1920) found no significant correlation between the number of days spent in broodiness and the annual egg production. The more frequent occurrence of broodiness during spring and summer months tends to reduce the importance of broodiness from the winter production standpoint. In this connection, the fact should be pointed out that, in the station flock of Rhode Island Reds, rate of production has been higher in the strains carrying a large percentage of broodiness than in the nearly broody-free strains. There is no evidence to indicate that there is any linkage between broody factors and rate factors, however. Combinations of high rate and non-broodiness appear to exist in the present flock.

The two-factor (A C theory) suggested by Goodale (1920) has been found to be substantiated by the inbreeding experiment. In brief, this theory assumes two dominant factors A and C for broodiness. Their combined action is necessary to produce broodiness. Males and females may carry either factor alone and only from those matings that bring together both factors will broody hens result.

Table V presented below gives the genetic composition of each breeding male and female together with the actual and expected proportion of broodies and non-broodies.

Table V indicates that the results fit expectations very well. Those families hatched during 1919 in which the

TABLE V
BROODINESS
1919 Hatch

Dam	Genetic Formula	Sire B3808 (Aa Cc)		
		Daughters		
		Actual	Expected	
B 357	Aa cc	0	.75 ± .45	Broody
		2	1.25	Nonbroody
B 359	Aa Cc (Broody)	4	4.5 ± .94	Broody
		4	3.5	Nonbroody
B 754	Aa cc	2	3.0 ± .92	Broody
		6	5	Nonbroody
B 699	Aa cc	2	1.86 ± .71	Broody
		3	3.14	Nonbroody
B 710	Aa cc	2	1.86 ± .71	Broody
		3	3.14	Nonbroody
B 785	Aa cc	2	1.86 ± .71	Broody
		3	3.14	Nonbroody
B1357	Aa cc	6	4.875 ± 1.17	Broody
		7	8.125	Nonbroody
B2219	aa cc	0	1 ± .59	Broody
		4	3	Nonbroody

1920 Hatch

Sire B5806 (aa Cc)		
B 357	Aa cc	0 1.25 ± .65 Broody
		5 3.75 Nonbroody
B3512	Unknown	No daughters

1921 Hatch

Sire B8273 (aa Cc)		
B8318	Aa cc	1 1 ± .59 Broody
		3 3 Nonbroody
B9796	Aa cc	0 .25 ± .29 Broody
		1 .75 Nonbroody
C23	aa CC	0 0 Broody
	aa Cc	3 3 Nonbroody
	aa cc	

Sire B8574 (aa Cc)		
B8318	Aa cc	1 1.25 ± .65 Broody
		4 3.75 Nonbroody
B9651	AA cc	1 1 ± .67 Broody
		1 1 Nonbroody
B9796	Aa cc	0 .5 ± .41 Broody
		2 1.5 Nonbroody

Sire B9275 (Aa Cc)		
B 357	Aa cc	1 .75 ± .46 Broody
		1 1.25 Nonbroody

number of broodies is less than expected may be explained by the fact that they were only carried until May. Some of the later maturing pullets would probably become broody later. In no case where the number of pullets tested amounts to six or more is there a deviation

beyond the probable error. Further work is being done on the question to test the validity of the theory.

WINTER RATE OF EGG LAYING

Rate as used in this report is calculated for each hen by dividing the number of eggs laid before March first of the pullet year by the number of days covered. High rate means 50 per cent. or more, or an average of at least one egg in two days during the winter season. Low rate is a percentage below fifty.

Evidence seems to indicate that two independent dominant factors R and R' are concerned in high rate. The presence of both is necessary to produce a rate of 50 per cent. or more. Either factor alone gives less than 50 per cent. rate. These two factors seem to be concerned with rate in the same way that factors A and C are concerned with broodiness.

TABLE VI
WINTER RATE OF PRODUCTION
1919 Hatch

Dam	Genetic Formula	Sire B3808 (Rr R'r')		
		Daughters		
		Actual	Expected	
B 357	RR R'r'	2	1.5 ± .41	High
		0	.5	Low
B 359	Rr R'r'	4	4.5 ± .94	High
		4	3.5	Low
B 754	Rr R'r'	5	4.5 ± .94	High
		3	3.5	Low
B 699	Rr R'r'	2	2.8 ± .74	High
		3	2.2	Low
B 710	RR R'r'	6	4.25 ± .77	High
		1	1.75	Low
B 785	RR R'R'	7	7	High
		0	0	Low
B1357	Rr R'r'	7	7.3 ± 1.20	High
		6	5.7	Low
B2219	RR R'r'	6	4.25 ± .77	High
		1	1.75	Low

1920 Hatch

Sire B5806 (Rr r'r')		
B 357	RR R'r'	3 2.5 ± .75 High
		2 2.5 Low
B3512	Unknown	No daughters' records

TABLE VI—Continued

1921 Hatch

			Sire B8273 (Rr R'r')
B8318	Rr R'r'	2	2.25 \pm .66 High
		2	1.75 Low
B9796	Rr R'r'	0	.56 \pm .33 High
		1	.44 Low
C23	rr r'r'	0	1 \pm .58 High
		4	3 Low

Sire B8574 (Rr R'r')

B8318	Rr R'r'	1	1.7 \pm .57 High
		2	1.3 Low
B9651	RR R'R'	2	2 High
		0	0 Low
B9796	Rr R'r'	1	.56 High
		0	.44 Low

Sire B9275 (RR R'R')

B 357	RR R'r'	2	2 High
		0	0 Low

Table VI gives the probable formula of each breeding hen and sire, also the actual and expected grouping of the daughters from each mating.

The agreement between the actual and theoretical grouping of the daughters for rate is rather close. The foundation hen B 357 appears to have been homozygous for R factor but heterozygous for R'. The impossibility of knowing the genetic make-up of males or females without a breeding test is well illustrated by the occurrence of "low rate" individuals throughout the experiment. By proper matings it should be entirely possible to establish strains of fowls breeding true for high rate.

GENETIC FORMULAE FOR FOWLS

The probable genetic formulae for the fowls used as breeders in this experiment are given below:

1919 Hatch

Sires					Dams				
B3808	Ee E'e	Mm Aa	Cc Rr	R'r'	B 357	Eo E'e'	mm Aa	cc RR	R'r'
					B 359	Eo E'e'	Mm Aa	Cc Rr	R'r'
					B 754	eo e'e'	Mm Aa	cc Rr	R'r'
					B 699	Eo E'E'	mm Aa	cc Rr	R'r'
					B 710	Eo E'e'	Mm Aa	cc RR	R'r'
					B 785	eo e'e'	mm Aa	cc RR	R'R'
					B1357	Eo E'e'	mm Aa	cc Rr	R'r'
					B2219	eo e'e'	mm aa	cc RR	R'r'

1920 Hatch

B5806	Mm aa Cc Rr r'r'	B 357	Eo E'e'	mm Aa cc RR R'r'
			B3512	..	Mm

1921 Hatch

B8273	Mm aa Cc Rr R'r'	B8318	...	mm Aa cc Rr R'r'
			B9796	...	mm Aa cc Br R'r'
			C23	...	Mm ... rr r'r'
B8574	Mm aa Cc Rr R'r'	B8318	...	mm Aa cc Br R'r'
			B9651	...	mm AA cc RR R'R'
B9275	..	mm Aa Cc RR R'R'	B9796	...	mm Aa cc Rr R'r'
			B 357	Eo E'e'	mm Aa cc RR R'r'

All blank spaces represent factors not determined by breeding tests. The above outline serves to show the complex of genetic factors concerned with winter egg production and makes clear the difficulty in establishing true-breeding flocks.

SUMMARY

Inbreeding reduces variability in winter egg production only when the foundation stock is largely homozygous for factors for heavy egg yield.

Sexual maturity seems to be retarded in many inbred pullets so that they are very slow in beginning to lay.

Body weight does not appear to be affected by inbreeding.

Winter egg yield shows a tendency to decline after the degree of inbreeding passes 25 per cent., but not necessarily so. A cumulative effect may be observed in succeeding generations.

Winter egg production probably depends on seven pairs of Mendelian factors. The development of a flock breeding true for these factors is entirely possible by proper methods.

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THE DISTRIBUTION OF CHROMOSOMES IN TETRAPLOID DATURAS

JOHN BELLING AND ALBERT F. BLAKESLEE

STATION FOR EXPERIMENTAL EVOLUTION, COLD SPRING HARBOR¹

THIS article is the second paper dealing, somewhat in detail, with the distribution of the chromosomes in *Daturas* with aberrant chromosome numbers, the first paper being that on triploids (3). A description of the microscopical methods used will be found in a short note in the AMERICAN NATURALIST (2).

Attraction of homologous chromosomes: At the late prophase and the metaphase of the first division in the pollen-mother-cells of true tetraploid *Daturas*, the chromosomes are, as a rule, arranged in connected sets of four each, the four chromosomes of each quadrivalent being of the same size (4). (Quadrivalents have been observed in tetraploid mosses by the Marchals (8)). The formula of such a tetraploid group is, $L_4 + 4l_4 + 3M_4 + 2m_4 + S_4 + s_4$, the letters representing the six size classes. A not uncommon arrangement is that of two rings connected at one side (lower right of Fig. 1, *l* and *m*), so as to form a figure of eight. In such quadrivalents, each chromosome is attached to one chromosome at one end, and to three chromosomes at the other end. Often, however, perhaps more often, the two rings are folded together (upper left of Fig. 1, *l* and *l*) so that each chromosome is attached to three others at each end. The homologous chromosomes may also form a single ring with two chromosomes attached at one side of it, so that one end of each of the two is free (*L* in Fig. 1). Several

¹ Paper read in abstract at the genetics section of the Botanical Society of America, December 27, 1922.

other arrangements are also found (such as *s* and *l* above the center of Fig. 1).²

Separation (disjunction) of chromosomes: The pulling apart of the quadrivalents is usually difficult to follow. In the observed cases of the double ring in one plane,

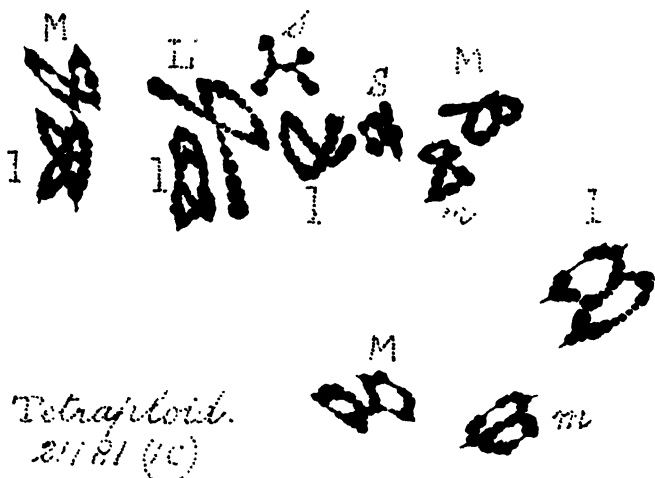


FIG. 1. Metaphase of the first division in a pollen-mother-cell of a tetraploid *Datura*. The preparation was fixed and stained in iron-acetocarmine. Subsequently the cytoplasm was pressed from the cell and adhered closely to the cover-glass. (This permitted the advantageous use of an apochromatic oil immersion objective of 1.4 aperture, with yellow-green light (2), and an immersed achromatic condenser of high aperture.)

The segmentation of each chromosome is visible. The four constituent chromosomes can be seen readily in eleven of the quadrivalents. There are 5 figures of eight (*l*, *M*, *M*, *m*, *m*), two double hoops (*l*, *l*), 2 examples of a ring with a V (*L*, *M*), one cross (*s*), one bent rod (*l*), and one quadrivalent (*S*) which is not readily classified.

two chromosomes went towards one pole and two towards the other, as was shown by the attachment of the fibers. The distributions of the chromosomes after the reduction division prove, however, that in some quadrivalents, three chromosomes must have passed to one pole and one to the other.

² Quadrivalents were not found by Gates (*Arch. f. Zellforsch.*, 1909), or by Davis (*Ann. Bot.*, 1911), in tetraploid *Oenothera lamarckiana*. They are difficult to demonstrate in the tetraploid *Primula sinensis*; but if several hundred well-fixed first metaphases are examined, and compared with those of the diploid form, it seems as if the majority of the 48 chromosomes were usually arranged in the tetraploid *Primula* in sets of two pairs each, and rarely 12 such sets may be counted.

Non-disjunction, single, double and triple: The chromosomes were counted after the reduction division, in the second metaphase, in more than 1500 pollen-mother-cells from true tetraploids. The first counts of the chromosomes of 24 tetraploid plants from different lines gave, in 218 pollen-mother-cells, 68 per cent. of 24 ∓ 24 distribution (Fig. 2), 30 per cent. of $23 + 25$ and 2 per cent. of $22 + 26$, with one case of $21 + 27$. This shows that non-

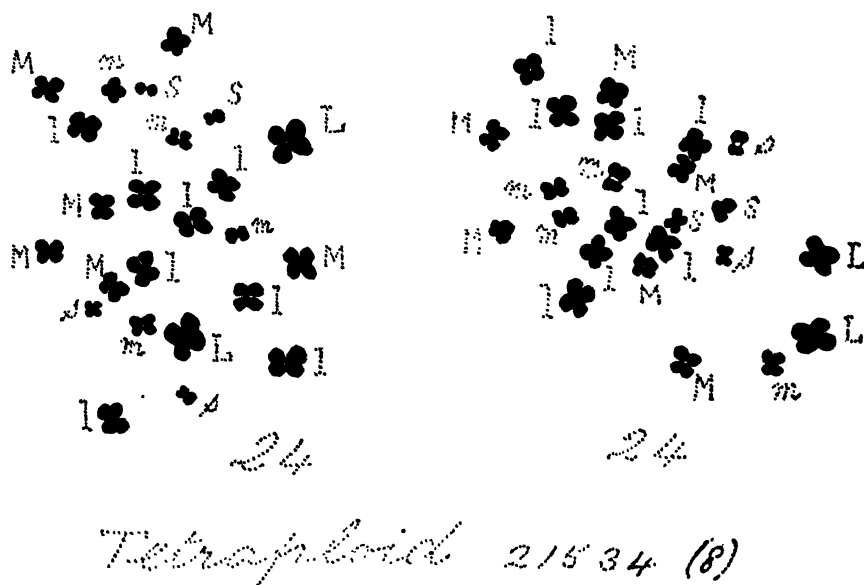


FIG. 2. Metaphases for the second division in the pollen-mother-cell. The preparation was made in the same way as that from which Fig. 1 was drawn with the camera. The sizes of the chromosomes are distinct whenever they are pressed into horizontality. The extra large (*L*), the small (*S* and *s*) and the small medium (*m*) are most easily distinguishable. The distinctions between *l* and *M*, and between *S* and *s*, are less easily made in this preparation.

disjunction is a regular phenomenon in tetraploid *Daturas*. (Gates observed a similar occurrence in tetraploid *Oenotheras* (6)). In order to study this further, one tetraploid plant was selfed, and the progeny (63 plants) grown to maturity. The chromosomes were counted in about twenty pollen-mother-cells, more or less, in all but one of these plants. The proportions of plants with different chromosome numbers are given in Table I.

TABLE I
NUMBERS OF CHROMOSOMES IN EACH OF 62 PLANTS OF THE SIBSHIP 21404

Numbers of chromosomes.....	48	49	47	(48)	50	46
Number of plants—						
27 (first lot)	25	2
35 (second lot)	30	3	1	1(?)
Totals	55	5	1	1
Calculated: (1) if only 2n pol- len is functional	47	8	8
(2) if 2n, (2n + 1), and (2n — 1) pollen functions ...	35	12	12	2	1	1

(In Table I, the 22-chromosome, or 26-chromosome, egg-cells are neglected in the calculation.)

The first lot in this table contains those seedlings potted out first from the seedpan; the second lot those of somewhat slower germination. There is an obvious advantage in having two groups to compare, even if no marked differences are noticeable.

If the chromosomes of the megaspores are distributed as are those of the pollen, then either the 24-chromosome egg-cells, or the 4n zygotes must evidently be more viable than the others. This has been found to happen in a (4n + 1) *Datura* investigated with regard to this point. (Van Overeem (9) has obtained somewhat similar results with the progeny of a tetraploid *Oenothera*).

The “(48)” plants in the table are pseudo-tetraploids, with 48 chromosomes, but with one set of three and one set of five.

All 48-chromosome plants in the sibship, having 20 or more than 20 pollen-mother-cells counted, are classified in Table II.³

With one exception, the deviations from 25 per cent. do not seem too great to be due to random sampling,

TABLE II
CLASSIFICATION OF 37 48-CHROMOSOME PLANTS FROM THE SIBSHIP 21404,
ACCORDING TO THEIR PERCENTAGES OF THE 23 + 25 DISTRIBUTION
OF CHROMOSOMES

Percentage	0	5	10	15	20	25	30	35	40	45	50	55	60
No. of plants ...	1	3	..	4	6	10	5	4	3			1	

³ The majority of the countings in sibship 21404 were done by Miss A. D. Bergner and Miss E. M. Lord.

since only a few more than 20 pollen-mother-cells were counted on the average in each plant. However, the plant with 59 per cent. of $23 + 25$ distribution has less than one chance in a thousand for its random occurrence. It is probably a pseudo-tetraploid and is therefore omitted from Table III.

TABLE III

DISTRIBUTION OF CHROMOSOMES IN 1379 POLLEN-MOTHER-CELLS OF THE 55 TRUE TETRAPLOID DATURAS IN SIBSHIP No. 21404

No. of plants	Chromosome groups	Distribution of chromosomes (Percentages)			Total No. of pollen-mother-cells
		24 + 24	23 + 25	22 + 26	
25	Single	75.5	21.8	2.7	404
30	Single	66.2	31.6	2.2	506
25	Double	73.6	24.7	1.8	284
30	Double	73.1	24.2	2.7	182

In the 1379 pollen-mother-cells in Table III there were two cases of $21 + 27$ chromosomes, and one of $20 + 28$. (The 22 cases of detachment (elimination) found, could not, of course, be included here; but will be discussed later.)

In Table III the distributions of chromosomes in the double groups from the two sets of early and late plants resemble one another closely, and are doubtless somewhat more reliable than the distributions resulting from the single groups. Hence, we may take the total of all the double groups in the true tetraploid plants of the sibship 21404 as forming the most reliable datum. This is 342 (73.2 per cent.) of $24 + 24$, 114 (24.4 per cent.) of $23 + 25$, 10 (2.1 per cent.) of $22 + 26$ and 1 (0.2 per cent.) of $21 + 27$.

If non-disjunction occurs with the same frequency in each quadrivalent, there would be a certain number of cases of double non-disjunction, both non-disjunctions being on the same side, thus giving a distribution of $22 + 26$ chromosomes. There would also be an equal number of cases of double non-disjunction on opposite sides (that is, double opposed non-disjunction), giving a distribution of $24 + 24$ chromosomes; each group con-

taining one set of one, and one set of three chromosomes, instead of two chromosomes to each set. Triple non-disjunction would give either $21 + 27$, or $23 + 25$; the latter distribution being three times as numerous as the former. Quadruple non-disjunction is probably negligible. Hence, if $x:1$ is the ratio of ordinary disjunction to non-disjunction in any quadrivalent (neglecting double non-disjunction in the same quadrivalent), the distribution of non-disjunction would, of course, be proportional to the terms of the binomial $(x + 1)^{12}$. Only the first four terms are important here. Reduced, they are x^3 , $12x^2$, $66x$, 220 . Adding to the first term the $33x$ cases of double opposed non-disjunction (which give the distribution $24 + 24$), and adding to the second term the 165 cases of triple opposed non-disjunction (which give $23 + 25$), we have the proportion in column 3, Table IV.

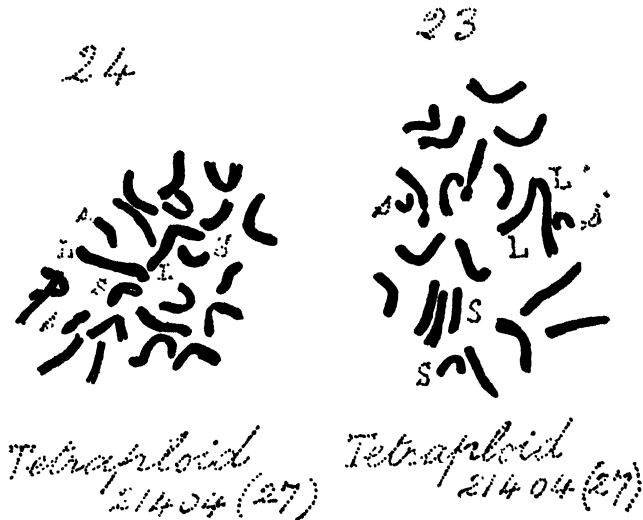
TABLE IV
CALCULATION OF CHROMOSOME DISTRIBUTION

Chromosome Distribution	Numbers Found	Theoretical Proportions	Numbers Calculated
$24 + 24$	342	$x^3 + 33x$	(342)
$23 + 25$	114	$12x^2 + 165$	115.5
$22 + 26$	10	$33x$	9.0
$21 + 27$	1	55	0.4
Total	467		Total 466.9

The third column in Table IV gives the theoretical proportions corresponding to the assumption that non-disjunction is the same in all 12 quadrivalents. By trial we get approximately 35 as the value of x , taking the first number as 342 and the total as 467. We then obtain for the second number, 115.5, for the third, 9.0, and for the fourth 0.4. From the fairly close agreement of the third and fourth numbers especially, we may assume that non-disjunction possibly takes place in each quadrivalent in about one thirty-sixth, or nearly three per cent. of the cases of possible disjunction.

From observation of the distribution of chromosomes after the reduction division in the pollen-mother-cells, we assume that about one quarter of the pollen-grains have 23 or 25 chromosomes. To test this, pollen-grains at the

stage of the first nuclear division were fixed and stained from a true tetraploid plant, No. 21404 (27). This plant had 25 per cent. of the $23 + 25$ distribution. Fifteen pollen-grains gave certain counts (Figs. 3 and 4).



FIGS. 3 and 4. Metaphases of the first division in the pollen-grain. The young pollen-grains were fixed and stained as before. The cells were somewhat flattened by the capillary pressure of the cover-glass. The line of the longitudinal division is distinct at the metaphase in the pollen-grains examined. This metaphase is obviously easier to count than the corresponding stage in the root-tips; but the second metaphase of the pollen-mother-cell is superior to both for counting, because of the shortness of the chromosomes.

TABLE V
CHROMOSOME DISTRIBUTION IN THE POLLEN-GRAINS

No. of chromosomes	24	23	25
No. of pollen-grains	11	3	1
Calculated	11.2	1.9	1.9

The numbers in Table V, though few, confirm the conclusions from the chromosome counts at the second metaphase in the pollen-mother-cells.

Detachment (elimination) of chromosomes: After the reduction division, one or two (rarely more) chromosomes are sometimes left detached between the two nuclei. These chromosomes often divide at the second division and form minute cells, microcytes, which perish (3). In the total of 1,401 pollen-mother-cells examined from the 55 tetraploid *Daturas* of the sibship 21404, there were

TABLE VI
DETACHMENT (ELIMINATION) OF CHROMOSOMES. NON-REDUCTION
Pollen tetrads of tetraploid plants. (Percentages)

Microspores..... Microcytes.....		4	4	4	4	4	2	Etc.	Percentage of detachment	No. of tetrads
		1	2	3	4					
Plant, and bud	Percentage of 23 + 25 distribution									
20587(1)	27	99.8					0.2		0.0	401
21107(1)	26	98.4	3.6	0.9				0.1	1.6	1002
21181(10)	27	96.7	1.0	2.2		0.1			3.3	827
21404(6)	18	93.8	4.6	1.7					6.3	416
21404(71)	25	98.0	0.5	1.2			0.3	0.1	1.8	1225
21404(80)a	27	98.2	0.9	0.9			0.1		1.7	940
21404(80)b	27	97.4	1.3	1.2					2.6	821
21536(8)	21	96.4	1.0	1.7			0.8	0.1	2.8	830
Percentages of totals.....		97.5	1.0	1.3		0.02	0.2	0.05	2.3	6462 (total)

22 observed cases of detachment of one or more chromosomes at the reduction division, which is 1.6 per cent. The total amount of detachment can be reckoned from the percentage of pollen tetrads which show microcytes (which are small cells containing the detached chromosomes). Table VI gives the numbers of microcytes seen in over 6,000 pollen tetrads from 7 true tetraploid plants. They show one rather excessive amount of detachment, namely, 6.3, and there is perhaps reason to suppose some effect of environment, as in triploids (3). The total percentage of detachment for the 6,462 tetrads is 2.3, which may not be significantly different from the 1.6 per cent. of detachment at the first division obtained by direct observation in 1,401 pollen-mother-cells. Any detachment at the second division may therefore be infrequent, as is also shown in triploids (3).

Non-reduction: The omission of the reduction division is rare in the tetraploids examined. A $48 + 48$ chromosome distribution results. The longitudinal division of each of the full number of chromosomes in the first metaphase plate without reduction has been observed more frequently in modified tetraploids with one or two extra chromosomes. The results of non-reduction, namely, two giant cells from each pollen-mother-cell, and giant pollen-

grains of double the ordinary volume, have also been observed in true tetraploids. Table VI shows that double-sized microspores were formed from about 0.2 per cent. of the pollen-mother-cells. There would be produced about half of this percentage of giant pollen-grains. From each of three plants which were apparently true tetraploids, 100 pollen-grains were measured. Out of these 300 grains, one alone had twice the average volume.

Chromosomes of functional egg-cells: Tetraploid *Daturas* were pollinated with pollen of diploids. The results are given in Table VII.

TABLE VII
CHROMOSOMES OF PROGENY OF TETRAPLOID *DATURAS* POLLINATED
BY DIPLOID

Numbers of chromosomes.....	$2n$	$2n + 1$	$3n - 1$	$3n$	$3n + 1$	$4n$
Numbers of plants.....	14	2	1	7	1	1
Calculated	1.1	6.8	1.1

The mode of origin of the $2n$ and $(2n + 1)$ plants in Table VII is unknown at present, but experiments to ascertain it are being undertaken. (The presence of one $4n$ plant may perhaps have been due to accidental selfing.) The remaining plants of the progeny apparently show that the megaspores also may have about 25 per cent. of the $23 + 25$ distribution of chromosomes after the reduction division, since a calculation on this basis fits closely to the facts.

Tetraploid (tetrasomic) inheritance: It may be shown (5) that the amount of non-disjunction occurring in a true tetraploid is not sufficient to cause a marked change in the Mendelian ratios for any particular pair of allelomorphs in the immediate offspring of a heterozygote. Since the general Mendelian results agree with expectation on the hypothesis of random assortment of the 4 chromosomes of a quadrivalent (7, 5); it follows, since each quadrivalent usually consists of two connected pairs, that the coming together of the members of these pairs must have been at random. The change of a true tetraploid to a double diploid would result from the two chromosomes of each of the two pairs having a preferential attraction.

Summary: (1) The chromosomes at the late prophase and metaphase of the first, or reduction division, in true tetraploid *Daturas*, are as a rule joined in quadrivalents, which in most cases are formed of two connected pairs.

(2) In each of the 79 true tetraploid plants examined there was a certain amount of the $23 + 25$ chromosome distribution after the reduction division. This averaged in the most reliable lot of counts, namely, the double groups from 55 sibs, nearly 25 per cent.

(3) The distribution of the chromosomes after the reduction division in true tetraploids, in the 467 cells with double groups, agreed closely with the distribution calculated for 35 cases of $2 + 2$ disjunction to 1 case of $3 + 1$ non-disjunction in the quadrivalents.

(4) Detachment (elimination) of chromosomes occurred in 2.3 per cent. of the pollen-mother-cells, chiefly (or wholly) at the reduction division. Non-reduction happened in 0.2 per cent. of pollen-mother-cells.

(5) On pollinating tetraploids by diploids, the proportions of $3n$, $(3n - 1)$, and $(3n + 1)$ progeny agreed with the hypothesis that there was approximately 25 per cent. of the $23 + 25$ distribution of chromosomes in the reduction division of the megaspore-mother-cells.

(6) When tetraploid *Daturas* are pollinated by diploids, there are produced also many diploid progeny, whose mode of origin is as yet unknown.

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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE. IX. NEW LIFE TABLES FOR *DROSOPHILA*¹

RAYMOND PEARL AND SYLVIA L. PARKER

IN the first of these Studies (27) we presented life tables for wild type and quintuple flies. These tables were based upon the collected data then available from the control portions of experiments in which these two sorts had been used. In the period which has elapsed since the publication of these pioneer life tables for *Drosophila* our work has been greatly extended, and in a number of particulars refined. Especially we have come to use in all the experimental work stocks which are more homogeneous *genetically*. Thus, for a long-lived stock we now use, instead of a random sample of a mass culture of wild type flies as was formerly the case, a random sample of our line 107, which is an inbred, long-lived "pure" strain. The origin of this line we have described in the second of these Studies (32). By "pure" we mean, of course, only that it is a highly inbred and homozygous strain. Similarly, we have come to use for a short-lived stock in experimental work pure vestigial strains, the study of Gonzalez (62) having shown that it is this mutant gene alone which is chiefly responsible for (or invariably associated with) the observed brachybioty of quintuple flies.

It seems desirable now to present new life tables for these genetically more homogeneous groups, in order that they may be at hand for reference in connection with further studies shortly to be published. Furthermore, we have not hitherto published any *Drosophila* life tables with age reckoned on a "centile of the equivalent life

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span" base, except in the case of wild type males from our original life tables.²

The actual observations on which the new life tables of this paper are based are presented in Table I. It will be seen that they include a total of 2,822 wild type flies of line 107, and 980 pure vestigial flies. The observations are recorded on a 6 day base unit in the case of wild type flies, and a 3 day base unit in the case of vestigials. We have found these units sufficiently fine for purposes of tabulation and derivative computation. The actual observations were made daily; the data as presented in Table I have been subsequently grouped.

TABLE I
OBSERVED DEATHS (d'_x) AND SURVIVORSHIP (l'_x) IN WILD TYPE LINE 107,
AND PURE VESTIGIAL FLIES

Age in days	Wild type, Line 107				Age in days	Pure Vestigial			
	Males		Females			Males		Females	
	d'_x	l'_x	d'_x	l'_x		d'_x	l'_x	d'_x	l'_x
1	18	1000	23	1000	1	1	1000	11	1000
7	12	987	16	984	4	48	998	43	979
13	26	979	34	972	7	81	893	43	897
19	85	960	86	941	10	86	715	57	815
25	99	900	86	888	13	81	526	55	706
31	132	829	96	827	16	51	349	63	601
37	187	736	159	759	19	33	237	47	481
43	173	603	152	647	22	35	164	51	391
49	206	480	162	539	25	22	88	27	294
55	285	333	275	425	28	5	39	35	242
61	82	131	156	230	31	4	29	30	176
67	62	72	99	120	34	3	20	17	118
73	36	28	64	50	37	3	13	14	86
79	3	3	6	5	40	2	7	14	59
85	1	1	1	1	43	1	2	4	32
—	—	—	—	—	46	—	—	6	25
—	—	—	—	—	49	—	—	4	13
—	—	—	—	—	52	—	—	2	6
—	—	—	—	—	55	—	—	1	2
Totals	1407	—	1415	—	Totals	456	—	524	—

In the graduation of the material the same plan was used as in the construction of the earlier life tables (*loc. cit.*).

The equations in the present case are as follows:

Wild Type, Line 107—Males;

$$\log l_x = e^{-.02282594x} (2.9999414 - .0674377x + .000677752x^2 - .00000369321x^3).$$

Wild Type, Line 107—Females;

$$\log l_x = e^{-.02541248x} (3.0000256 - .0761521x + .0000854457x^2 - .00000449705x^3).$$

Vestigial—Males;

$$\log l_x = e^{-.08729445x} (2.9961959 - .1959626x + .00456415x^2 - .0000377354x^3).$$

Vestigial—Females;

$$\log l_x = e^{-.08010007x} (3.0019283 - .1188025x + .00165311x^2 - .00000842816x^3).$$

² Cf. Pearl (61), and Pearl and Doering (63).

The observations and fitted lines are shown in Fig. 1.

As a whole the fits are reasonable, if one has as an objective simply the general sweep of the observations and is not concerned, as the actuary is, to represent every

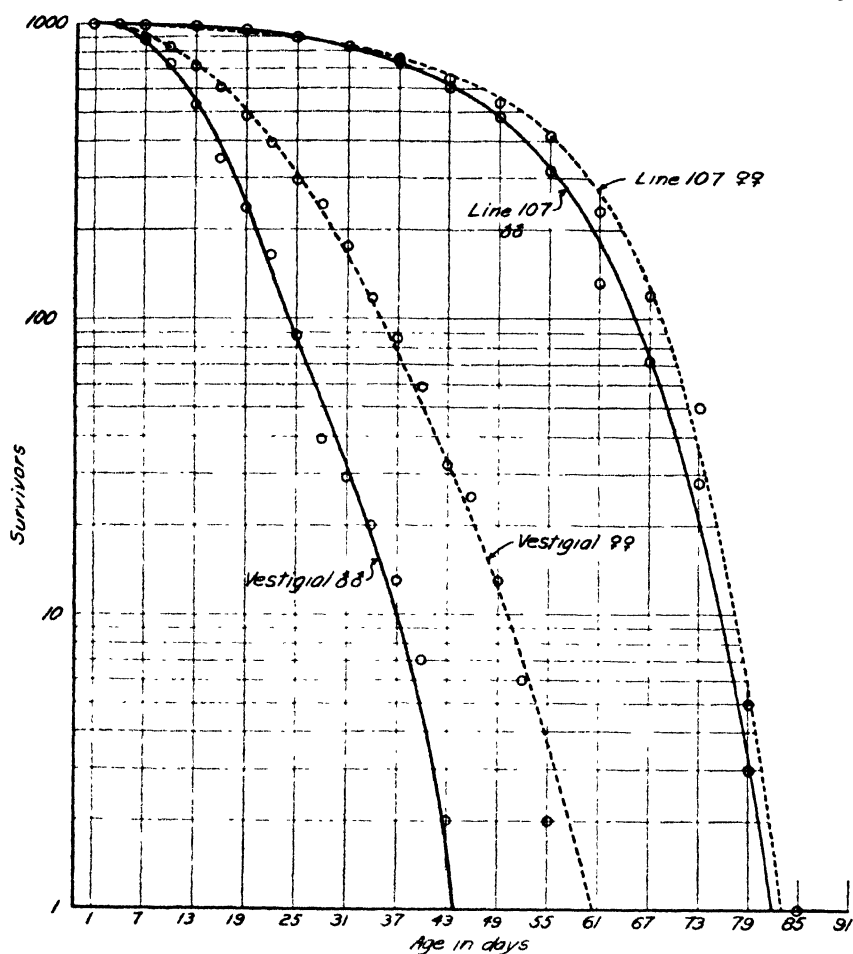


FIGURE 1

Diagram showing the observed and graduated l_x points for (a) line 107 wild type, and (b) vestigial flies. The small circles are the observations from Table I, and the smooth lines the fitted curves from the equations.

fluctuation in the curve. From this point of view the line 107 curves are entirely satisfactory. The vestigial curves are close fits up to 31 days in the females and 49 days in the males. The upper tails of both vestigial curves are bad fits, underestimating the observations in the females and overestimating in the males. To take

care of these end observations would require additional constants in the equations. But for all purposes to which fly life tables are ever likely to be put the present graduations will probably be adequate.

The complete life tables are presented in Tables II, III, IV and V.

From these tables the following points are to be noted:

(1) As compared with the genetically more heterogeneous earlier life tables, the purer strains of the present tables exhibit (a) a greater expectation of life at emergence in both sexes of line 107, but a shorter total life span than in the general wild type population; (b) substantially the same expectation of life at emergence and total life span, in male vestigials as in male quintuples; and (c) a distinctly longer expectation of life at emergence and longer total life span, in female vestigials than in female quintuples.

(2) These tables show the same relation between sexes in respect of mortality that human life tables do. The females have lower q_x values (deathrates) than do the males, throughout life. The sex differences in mortality are much more pronounced in the vestigials than in the wild type line 107.

(3) The form of the vestigial life curve is distinctly different from that of the wild type flies. The vestigial mortality is characterized by a plateau of nearly constant q_x values in middle life (in the males forming even a slight dip convex to the base). This phenomenon gives the vestigial l_x curves their peculiarly flattened appearance in the middle portion of their course.

It is desirable to compare these new *Drosophila* life tables with each other and with the human tables by putting the ages upon a relative base, using as a unit a centile (a hundredth part) of the equivalent life span, in the manner described by Pearl (61). The data of Tables II to V are transferred to a centile age basis in Table VI.

In Fig. 2 these centile distributions for *Drosophila* are compared with similar data from (a) human life tables (Glover (51)), and (b) the saturniid moth *Telea polyph-*

TABLE II

LIFE TABLE FOR DROSOPHILA—WILD TYPE. LINE 107—MALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	0.2	45.8	46	551	43.5	12.3
2	1000	0.6	44.8	47	527	46.6	11.8
3	999	1.0	43.8	48	502	49.8	11.3
4	998	1.3	42.9	49	477	53.2	10.9
5	997	1.7	41.9	50	452	56.9	10.4
6	995	2.0	41.0	51	426	60.8	10.0
7	993	2.4	40.1	52	400	65.0	9.6
8	991	2.8	39.2	53	374	69.5	9.2
9	988	3.2	38.3	54	348	74.2	8.8
10	985	3.5	37.4	55	322	79.2	8.4
11	981	3.9	36.5	56	297	84.5	8.0
12	978	4.3	35.7	57	272	90.2	7.7
13	973	4.7	34.8	58	247	96.2	7.4
14	969	5.1	34.0	59	223	102.5	7.0
15	964	5.5	33.2	60	200	109.2	6.7
16	958	6.0	32.3	61	179	116.3	6.4
17	953	6.4	31.5	62	158	123.8	6.1
18	947	6.9	30.7	63	138	131.7	5.9
19	940	7.4	29.9	64	120	139.9	5.6
20	933	7.9	29.2	65	103	148.8	5.3
21	926	8.5	28.4	66	88	157.9	5.1
22	918	9.0	27.6	67	74	167.6	4.9
23	910	9.6	26.9	68	62	177.7	4.7
24	901	10.3	26.1	69	51	188.3	4.4
25	892	10.9	25.4	70	41	199.4	4.2
26	882	11.7	24.6	71	33	211.0	4.1
27	872	12.4	23.9	72	26	223.1	3.9
28	861	13.3	23.2	73	20	235.8	3.7
29	849	14.1	22.5	74	15	248.9	3.5
30	837	15.1	21.8	75	12	262.6	3.4
31	825	16.1	21.1	76	9	276.8	3.2
32	811	17.2	20.5	77	6	291.5	3.1
33	798	18.3	19.8	78	4	306.7	2.9
34	783	19.5	19.1	79	3	322.5	2.8
35	768	20.8	18.5	80	2	338.7	2.6
36	752	22.3	17.9	81	1	355.5	2.4
37	735	23.8	17.3	82	1	372.7	2.2
38	717	25.4	16.7				
39	699	27.2	16.1				
40	680	29.1	15.5				
41	660	31.1	14.9				
42	640	33.3	14.4				
43	619	35.5	13.8				
44	597	38.0	13.3				
45	574	40.7	12.8				

TABLE III

LIFE TABLE FOR DROSOPHILA—WILD TYPE. LINE 107—FEMALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	0.6	48.0	46	619	30.7	14.2
2	999	1.1	47.1	47	600	33.0	13.6
3	998	1.5	46.1	48	580	35.6	13.0
4	997	2.0	45.2	49	560	38.4	12.5
5	995	2.5	44.3	50	538	41.4	11.9
6	992	2.9	43.4	51	516	44.7	11.4
7	990	3.3	42.5	52	493	48.3	10.9
8	986	3.7	41.6	53	469	52.3	10.4
9	983	4.2	40.8	54	444	56.5	9.9
10	978	4.5	39.9	55	419	61.1	9.5
11	974	4.9	39.1	56	394	66.1	9.0
12	969	5.3	38.3	57	368	71.4	8.6
13	964	5.7	37.5	58	341	77.3	8.2
14	959	6.0	36.7	59	315	83.4	7.8
15	953	6.4	35.9	60	289	90.2	7.4
16	947	6.7	35.2	61	263	97.3	7.0
17	940	7.1	34.4	62	237	105.0	6.6
18	934	7.4	33.6	63	212	113.2	6.3
19	927	7.7	32.9	64	188	122.0	6.0
20	920	8.0	32.1	65	165	131.3	5.7
21	912	8.4	31.4	66	144	141.3	5.4
22	905	8.7	30.6	67	123	152.0	5.1
23	897	9.1	29.9	68	105	163.2	4.8
24	889	9.4	29.1	69	87	175.1	4.6
25	880	9.8	28.4	70	72	187.7	4.4
26	872	10.2	27.7	71	59	201.0	4.1
27	863	10.6	27.0	72	47	215.0	3.9
28	854	11.0	26.2	73	37	229.7	3.7
29	844	11.5	25.5	74	28	245.2	3.5
30	835	12.0	24.8	75	21	261.3	3.3
31	825	12.5	24.1	76	16	278.3	3.2
32	814	13.1	23.4	77	11	295.9	3.0
33	804	13.7	22.7	78	8	314.4	2.8
34	793	14.4	22.0	79	6	333.4	2.7
35	781	15.2	21.3	80	4	353.2	2.5
36	769	16.0	20.6	81	2	373.6	2.4
37	757	17.0	19.9	82	1	394.7	2.2
38	744	18.0	19.3	83	1	416.6	1.9
39	731	19.1	18.6				
40	717	20.3	17.9				
41	702	21.7	17.3				
42	687	23.1	16.6				
43	671	24.8	16.0				
44	655	26.6	15.4				
45	637	28.5	14.8				

TABLE IV
LIFE TABLE FOR *DROSOPHILA*—VESTIGIAL—MALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	0.0	14.1	26	72	162.7	5.8
2	1000	9.0	13.1	27	61	162.5	5.7
3	991	18.1	12.2	28	51	162.0	5.6
4	973	27.4	11.7	29	43	161.1	5.5
5	946	36.7	10.7	30	36	160.8	5.4
6	912	45.8	10.1	31	30	160.7	5.3
7	870	55.4	9.6	32	25	161.5	5.1
8	821	64.7	9.1	33	21	163.6	4.9
9	768	73.8	8.6	34	18	167.7	4.6
10	712	82.8	8.2	35	15	174.5	4.4
11	653	91.5	7.9	36	12	184.8	4.1
12	593	100.0	7.6	37	10	198.5	3.8
13	534	108.1	7.3	38	8	219.6	3.4
14	476	115.9	7.0	39	6	246.0	3.1
15	421	123.1	6.8	40	5	279.6	2.8
16	369	129.9	6.7	41	3	320.9	2.5
17	321	136.2	6.5	42	2	370.4	2.3
18	277	141.8	6.4	43	1	427.7	2.0
19	238	146.8	6.3	44	1	491.9	1.7
20	203	151.2	6.2				
21	172	154.8	6.1				
22	146	157.8	6.0				
23	123	160.0	6.0				
24	103	161.5	5.9				
25	86	162.4	5.9				

mus, on the basis of our calculations of an ungraduated life table from data as to the duration of life of this form in the adult stage given by the Raus (64).

From these data it appears that:

(1) The distribution of mortality in the different parts of the biologically equivalent life span is substantially *identical* quantitatively in an inbred strain of *Drosophila* (line 107) and in human beings of the present time. That is to say, if we take as our base line biological age, mortality is distributed along that base line in quantitatively the same manner in man and a particular inbred strain of wild type *Drosophila*. This does not, of course, in the least warrant the assertion that the forces determining rates of mortality in the two cases are identical. In detail they obviously are not. So far as is known, for example, the tubercle bacillus is not pathogenic to *Droso-*

TABLE V

LIFE TABLE FOR DROSOPHILA—VESTIGIAL—FEMALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	7.7	19.8	36	88	124.9	7.1
2	992	11.0	19.0	37	77	127.6	6.9
3	981	14.3	18.2	38	67	130.3	6.8
4	967	17.7	17.4	39	58	133.1	6.7
5	950	21.2	16.7	40	50	135.8	6.5
6	930	24.7	16.1	41	44	138.6	6.4
7	907	28.2	15.5	42	38	141.4	6.3
8	882	31.7	14.9	43	32	144.3	6.1
9	854	35.3	14.3	44	28	147.3	6.0
10	824	38.8	13.8	45	24	150.4	5.9
11	792	42.4	13.3	46	20	153.6	5.7
12	758	46.0	12.9	47	17	157.0	5.6
13	723	49.6	12.5	48	14	160.7	5.4
14	687	53.2	12.1	49	12	164.7	5.3
15	651	56.8	11.7	50	10	169.0	5.1
16	614	60.4	11.3	51	8	173.6	5.0
17	577	64.0	11.0	52	7	178.7	4.8
18	540	67.6	10.7	53	6	184.3	4.7
19	503	71.1	10.4	54	5	190.5	4.5
20	467	74.7	10.1	55	4	197.3	4.3
21	432	78.1	9.8	56	3	204.9	4.1
22	399	81.6	9.6	57	2	213.3	3.9
23	366	85.0	9.3	58	2	222.6	3.7
24	335	88.4	9.1	59	1	232.9	3.5
25	305	91.7	8.9	60	1	244.3	3.2
26	277	95.0	8.7	61	1	256.8	2.9
27	251	98.2	8.5				
28	226	101.4	8.3				
29	203	104.5	8.1				
30	182	107.5	8.0				
31	163	110.5	7.8				
32	145	113.5	7.6				
33	128	116.4	7.5				
34	113	120.1	7.3				
35	100	122.2	7.2				

phila. The facts only mean that different in their *qualitative* details as are the lethal forces which attack the two organisms, man and a certain kind of *Drosophila*, they are alike in their *quantitative* relations to biological age.

(2) In another kind of *Drosophila*, differing so far as is known from the kind mentioned in the previous paragraph *only* in respect of one single second chromosome gene (and its somatic expression), the distribution of mortality in respect to biological age is widely *different*

TABLE VI

SURVIVORSHIP DISTRIBUTIONS OF *DROSOPHILA* BY CENTILES OF LIFE SPAN

Centiles of Equivalent Life Span	Line 107 ♂	♀	Vestigial ♂	♀	Centiles of Equivalent Life Span	Line 107 ♂	♀	Vestigial ♂	♀
0	1000	1000	1000	1000	50	654	689	137	168
1	1000	1000	1000	996	51	637	676	127	157
2	999	999	1000	991	52	620	662	118	146
3	999	998	998	984	53	602	648	110	136
4	998	996	994	976	54	583	634	102	127
5	997	994	989	968	55	564	619	94	118
6	996	992	982	958	56	545	604	87	110
7	994	990	973	947	57	526	588	81	102
8	992	988	963	935	58	506	571	75	94
9	990	985	951	922	59	486	554	70	87
10	988	982	938	909	60	466	537	65	80
11	985	979	923	894	61	446	518	60	74
12	982	975	907	878	62	426	499	56	68
13	979	971	889	861	63	405	480	52	63
14	976	967	870	844	64	384	460	48	58
15	973	963	851	826	65	363	440	44	53
16	969	958	830	807	66	342	419	41	49
17	965	953	808	788	67	321	398	38	45
18	961	948	785	768	68	300	377	35	41
19	957	943	762	748	69	278	356	32	37
20	952	938	738	727	70	258	334	30	34
21	947	933	713	706	71	240	312	28	31
22	942	927	688	685	72	221	290	26	28
23	936	921	663	663	73	202	269	24	26
24	930	915	638	641	74	184	248	23	24
25	924	909	613	619	75	167	227	21	22
26	918	903	587	597	76	150	207	19	20
27	911	896	561	575	77	135	188	18	18
28	904	889	536	553	78	120	169	17	16
29	897	882	511	530	79	106	151	15	14
30	890	875	486	508	80	94	134	14	13
31	882	868	462	487	81	82	118	13	12
32	874	861	439	466	82	72	103	12	11
33	865	854	416	445	83	62	89	11	9.4
34	856	846	393	425	84	53	76	10	8.4
35	847	838	371	405	85	45	64	9.2	7.6
36	837	830	350	386	86	38	54	8.4	6.8
37	827	822	330	367	87	31	45	7.6	6.0
38	816	814	311	348	88	26	37	6.9	5.4
39	805	805	292	330	89	21	30	6.2	4.8
					90	17	24	5.5	4.2
40	794	796	274	312	91	14	19	4.8	3.7
41	782	787	257	295	92	11	15	4.2	3.2
42	769	777	240	278	93	8.3	11	3.7	2.8
43	756	767	225	263	94	6.4	8.3	3.2	2.4
44	743	757	210	248	95	4.8	6.1	2.7	2.1
45	730	747	196	233	96	3.6	4.5	2.3	1.8
46	716	736	182	219	97	2.6	3.2	1.9	1.6
47	701	725	170	206	98	2.0	2.2	1.6	1.4
48	686	713	159	193	99	1.4	1.4	1.3	1.2
49	670	701	148	180	100	1.0	1.0	1.0	1.0

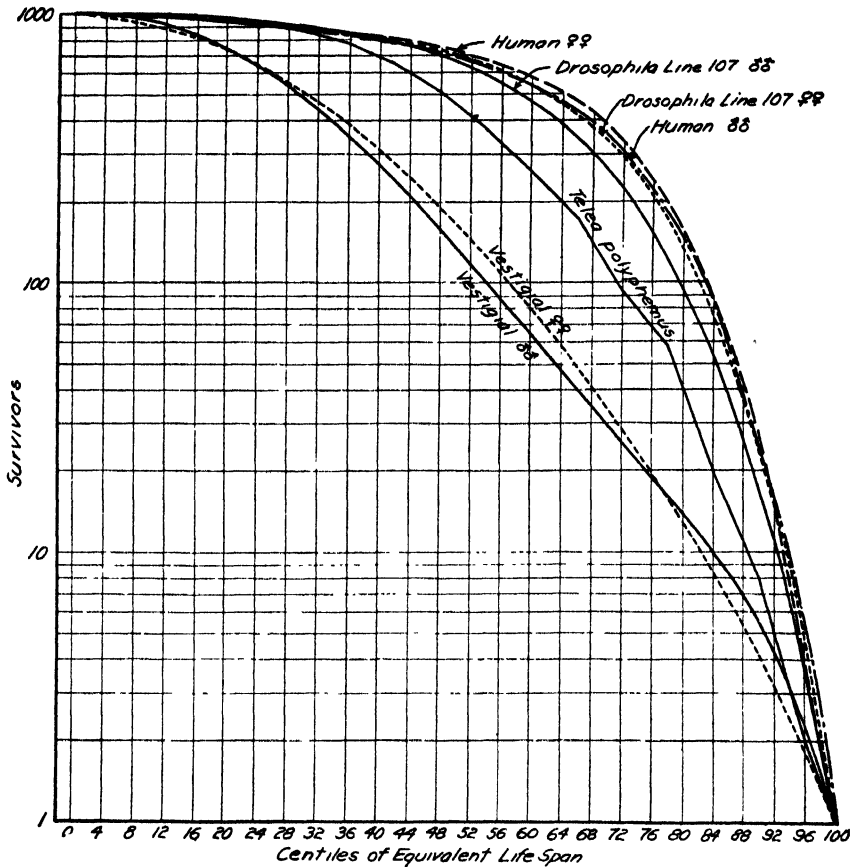


FIGURE 2

Comparing the survivorship distributions of (a) *Drosophila* line 107 males; (b) *Drosophila* line 107 females; (c) *Drosophila* vestigial males; (d) *Drosophila* females; (e) human males; (f) human females; (g) *Teia polyphemus*, both sexes together, over the equivalent life spans.

quantitatively from that found either in man or in wild type *Drosophila*, even in spite of the fact that both kinds of *Drosophila* spent their entire lives in statistically identical environments, so far at least as concerns temperature, optimum population density and housing, food, season and climate. This fact seems to us quite as significant as the identity established in the preceding paragraph. It shows that a unit change in the genetic constitution of an organism may not only be associated with a marked alteration of the absolute length of the life span, but also with a profound alteration of the form of the life curve.

(3) Wild type *Drosophila* and vestigial are plainly approximating two quite distinct theoretically possible forms of life curves. One of these types, which may be called the rectangular, would in the limit show all the individuals starting at birth together living to the same age and then all dying together at the same time. q_x would equal zero up to this "day of judgment," and then would on that day take the extreme value of 1,000 (on a per-thousand base). The closest approximation yet seen in living nature to the theoretical limit is *Proales*, as set forth by Pearl and Doering (63); the next closest approximation yet described is that of *Drosophila* line 107 and present day human beings, as shown in Fig. 2 above.

The other theoretical type of life curve which concerns the present discussion may be called the diagonal. This, in the limit, would be a case where the instantaneous death rate q_x would be constant at all ages from the start at birth to the demise of the last survivor. Plotted on arithlog paper the l_x line would be a straight diagonal line. The closest approach yet found in living nature to this theoretical type of life curve is that given by vestigial *Drosophila*, as shown in Fig. 2. All other life curves yet known fall between the rectangular and diagonal types.

There is a third type theoretically possible, but not actually realized in experience as yet. This is the case in which q_x has very large values in early ages, and thereafter nearly constant values until the last survivor is reached. This would mean an l_x line which dropped sharply to a low level in the earliest ages and then ran along a nearly horizontal course to the end of the life span of the last survivor. This would be the life curve of a very heavy selective mortality of early life. It is difficult to see how it could occur in a population genetically homogeneous in respect of factors influencing duration of life. But it could readily occur in a population genetically mixed relative to these factors.

LITERATURE CITED

(The plan of numbering citations is explained in the second of these Studies, AMER. Nat., Vol. 56, p. 174.)

62. Gonzalez, B. M. Experimental Studies on the Duration of Life. VIII. The Influence upon Duration of Life of Certain Mutant Genes of *Drosophila melanogaster*. AMER. NAT., Vol. 57, pp. 289-328. 1923.
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BOOKS AND LITERATURE

A BIBLIOGRAPHY OF FISHES

DR. BASHFORD DEAN'S third and completing volume of "A Bibliography of Fishes," which has just been distributed by the American Museum of Natural History, will be considered with a feeling of great satisfaction by everyone who has an interest in any of the numerous and diverse subjects relating to fishes and fisheries.

The first two volumes of nearly 1,500 pages contain a bibliography arranged alphabetically by authors. It includes about 40,000 titles. Without the third volume the first two would be complete in themselves, but the use of them requires as a prerequisite a knowledge of author's names and subjects. Such knowledge the specialist has to a greater or less degree of completeness, though even in his own field memory often fails him. Consequently, the third volume is the most useful of them all, for its most important part is an index arranged under subject headings, and as it refers back to the other two volumes it serves as a key to them and makes a unit of the whole.

This invaluable work has been in preparation for thirty years. During this time Dr. Dean has had at least a dozen collaborators. The first two volumes were under the editorship of the late Dr. C. R. Eastman, who was succeeded by Dr. E. W. Gudger with the cooperation of Mr. A. W. Henn.

It might well serve as a model for a bibliography of each of the vertebrate classes. It is doubtful, however, if the bibliography of any other class would include so many titles, or so many names of illustrious authors, containing as it does such a large proportion of the greatest zoologists of all times.

Not only will the men interested in fishes be under great obligations to Dr. Dean and his colleagues, but comparative anatomists will be also, for the anatomy of the primitive vertebrates is fundamental to an understanding of all anatomy.

The literature relating to any one subdivision of zoology has grown so great in magnitude that no one of average memory can hope to keep in mind even that part of it relating to his own special field; and who does not more than occasionally wish to go afield?

The first caption of the subject index is *abdominal pores*, under which are many references, some of which are starred to indicate special importance. Besides this, there is a very adequate digest of the subject, including views of different authors

and probable homologies. This subject happens to be of present interest to the writer, for recently he wished to refer a student in comparative anatomy to the literature relating to abdominal pores in the Elasmobranchs and found his knowledge limited to the text-books. These, naturally having to cover the entire field of comparative anatomy, could not give more than the fair proportionate space of a few lines to this small part of it. This is cited as an instance of the immediate usefulness of the book. Scattered through the subject index are interpretations and notes of information.

Glancing hastily through the index the heading, *air-bladder*, catches the eye, with sub-heads covering development, homologies, anatomy, ducts, degeneration, specialization, gaseous content, functions and the conditions of the air-bladder in the different groups of fishes. In this way each part of anatomy is taken up, and each treated in the same exhaustive manner. The skeleton, for instance, is treated under its separate parts, as skull or pectoral girdle, and each followed through the separate fish groups. Under angling, general treatises are separated in different languages; history of angling is included; early laws, different methods of angling for different fishes and in different regions. Aquaria and aquarium fishes are very fully treated with long lists of papers on aquarium methods and on specific fishes.

Selecting a few of the headings at random one finds: color, behavior, commensalism, digestion, ecology, electric organs, functions, sex determination, faunas considered geographically, products and by-products, food of fishes and fishes as food, legislation and protection, preserving methods, the use of poison in capturing, reproduction and sound production. These are only a few of the diverse headings, but enough to show the scope of the work.

The subject index occupies only half of the third volume. This condensing is possible because the papers noted in the author's index are referred to by a scheme of abbreviations.

In the preface of the first and third volumes will be found a history of the work from its beginning. A very attractive feature lies in the quaint quotations from the old authors on the pages next to the title of the same volumes. In the selection of them we recognize the hand of our old friend, Dr. Dean. The writer may here take occasion to thank him and his collaborators for the bright light thrown on his chosen pathway by the bibliography of fishes.

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SHORTER ARTICLES AND DISCUSSION

RADIUM RADIATIONS AND CROSSING OVER

DURING the summer of 1917 while at the Marine Biological Laboratory at Woods Hole, I made a series of preliminary tests of the effect of radium radiations on crossing over in the second chromosome of *Drosophila*. The radium used—50 mg. of the pure bromide—was very kindly loaned by Dr. Charles Packard, who was studying its effect on embryological processes.¹ Although there was clear evidence of a change in the percentage of crossing over, the results as a whole seemed contradictory, and publication of them was reserved until further tests could be made. There has been no opportunity to do this as yet, but the recent work of Mavor² and Mavor and Svenson³ on the effect of X-rays on crossing over seems to place these data in a somewhat different light. For that reason a brief account of the results appears to be advisable.

The methods were similar to those used in my tests of the effect of temperature on crossing over⁴ and followed more recently by Mavor. Five newly hatched females heterozygous for the second chromosome genes for black-purple-curved were subjected to radium radiations for each of the different intervals subsequently noted, and five sister individuals were reserved as controls. A glass tube about 1 by $\frac{1}{4}$ inches containing the flies was placed in contact with the glass tube containing the radium. The alpha particles and some of the slower beta rays were thus cut off by the glass, but most of the beta rays and all the gamma rays may be supposed to have acted on the flies.¹ Since the flies were constantly moving about in the tube each received an approximately equal radiation. Both the experimentally treated and the control females were back-crossed to black-purple-curved males, and placed in bottles which were changed every four days. Counts of the offspring were made for a period of twelve days only.

¹ *Biol. Bull.*, xxxv, 1. 1918.

² *Proc. Soc. Exp. Biol. and Med.*, xx, p. 335. 1923.

³ *Sci.*, lviii, 1494. 1923.

⁴ *Jour. Exp. Zool.*, 24, 2. 1917.

The following table gives the percentages of crossing over shown by the different sets of flies for the black-purple region only. This shorter region has been shown to be more "sensitive" to temperature changes than the purple-curved, and the values for the latter region in the radium series parallel the ones given with less significant differences.

SUMMARY OF THE EFFECTS OF RADIUM RADIATION ON CROSSING OVER

Percentages of crossing over for black-purple

Days after mating	Control	Radium 20 min.	Radium 20 min. at same time on 2 successive days	Radium 40 min.
1-4	7.60 \pm 0.74	5.02 \pm 0.95	7.52 \pm 0.88	6.86 \pm 1.05
5-8	7.58 \pm 0.75	4.57 \pm 0.62	9.52 \pm 2.16	8.49 \pm 1.53
9-12	3.60 \pm 0.48	5.30 \pm 0.96	3.91 \pm 0.98	9.50 \pm 1.33

In addition to the periods of radiation given a set was exposed to radium for one hour, but this severe exposure had a very marked lethal effect on the eggs laid. The number of flies hatched—less than 100 in 12 days—was too small to give any significant crossing over values. The same effect, to a lesser degree, was noted in the 40 minute experiment. Several tests of flies from these two series showed that they were themselves sterile, as previously reported by Packard.⁵

In general it will be seen that radiation causes an increase in crossing over which is most extreme for the most severe radiation. After a 40 minute exposure this effect appears first between the 5th and 8th day, and shows most plainly in the 9-12 day period. The difference here is 5.9 per cent., while the probable error of the difference is ± 1.41 per cent. The females which received a 20 minute exposure at the same time on two successive days showed an apparent increase in the 5-8 day period, but the probable error is so high as to make this of doubtful significance.

The result of the exposure for 20 minutes is of interest, though the data are too meager to admit of positive conclusions. The values for both the first and the second 4 day periods show a possibly significant *decrease* in the percentages of crossing over. This is especially true of the second value which is 3.01 per cent. less than the control, and the probable error of the difference is .97 per cent. The third value shows a slight but perhaps signifi-

⁵ Jour. Exp. Zool., 19, 3. 1915.

cant increase. It would appear that a short exposure to radium radiations produces a decrease in crossing over in those eggs which are closer to the point where the crossing over takes place, but a slight increase in those which are still further back in the early oogonial period.

There is an interesting parallelism between the effects noted above, and the first chromosome data of Mavor following X-ray exposure. I have shown⁶ that this chromosome appears to cross over less freely than at least the black-curved section of chromosome II. Mavor and Svenson found that the same dose of X-rays which produced an increase in crossing over in chromosome II caused a decrease in chromosome I. Since the most effective agents in radium radiation are apparently the gamma rays, which are actually hard X-rays, one might venture the suggestion that the same dose of X-rays which produces the marked increase in crossing over in the second chromosome, produces only the initial decrease in chromosome I because it responds less freely. In any case these data make it of interest to know the effect on crossing over of varying amounts of X-ray or radium treatment.

It is obvious that radium exposure corresponds with the X-ray treatment in the fact—pointed out by Mavor and Svenson—that it produces some change in the protoplasm, or the chromatin, of cells which have not reached the oocyte stage. I calculated that approximately 150 eggs were in this stage or later in the ovaries of female flies which had just hatched. The effect of X-rays for 3 minutes or radium for 20 minutes is thus produced on eggs in the oogonial stage, though it does not become apparent until the period of crossing over is reached. It should be noted, however, that temperature also produces an effect on oögonia, for I showed that only eggs which have been exposed to high temperature for at least 24 hours previous to the time of crossing over, show a change in the crossing over percentage.

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BIRD MIGRATION AND PLUMAGE SUCCESSION CHARTS

AMONG the many complex phenomena of bird life, the distribution and seasonal movements of the various migrational groups within any given area and the annual procession of plumages,

⁶ *Jour. Exp. Zool.*, 32, 2. 1921.

with their associated molts, cause, among beginning students of the subject, perhaps the most confusion. The charts accompanying this paper have been used with much success by the writer, with such students.

Within any given area (represented by the stippled portion of Fig. 1) there normally occur six more or less well-defined groups of birds. (1) Permanent residents, or those which apparently do not migrate, at least to any great degree. This group contains such birds as are represented the year around *as to species*, but not necessarily as to individuals, *i.e.*, it is probable that the individuals that one sees in the winter are not the same ones observed during the nesting season, in most cases. Familiar eastern examples of birds of this group are: white-breasted nuthatch (*Sitta carolinensis*), quail (*Colinus virginianus*), downy woodpecker (*Dryobates pubescens medianus*) and hairy woodpecker (*Dryobates villosus*). (2) Spring migrants, or those birds which nest to the north of the area and winter to the south of it, and pass through the area northward in early spring. Examples: white-throated sparrow (*Zonotrichia albicollis*), myrtle warbler (*Dendroica coronata*) and yellow-bellied flycatcher (*Empidonax flaviventris*). (3) Fall migrants, or those birds of the group just mentioned, which are returning to their wintering grounds in the fall. (4) Summer residents, the largest group, consisting of those birds which winter to the south of the area, and return to it each season to nest. This group contains the best known birds of each area, since they pass a longer time within its bounds than do the birds of any other group, beside nesting and rearing their progeny here. Such birds are the familiar robin (*Planesticus migratorius*), bluebird (*Sialia sialis*) and a host of others quite as well known. (5) Winter residents, comprising those birds which nest to the north of the area, and winter within it, such as: Red-breasted nuthatch (*Sitta canadensis*), bluebill, or scaup duck (*Marila affinis*) and herring gull (*Larus argentatus*). (6) Irregular visitants, which are not, properly, members of the avifauna of the area, but accidental visitors, which straggle in and out in an unpredictable fashion. Such an irregular visitant, for our northeastern states, is the evening grosbeak (*Hesperiphona vespertina*). In Figure 1 the spring migration is shown by broken lines, and the fall migration by solid lines. The arrows for the irregular visitant group are intended to indicate that their movements are capricious.

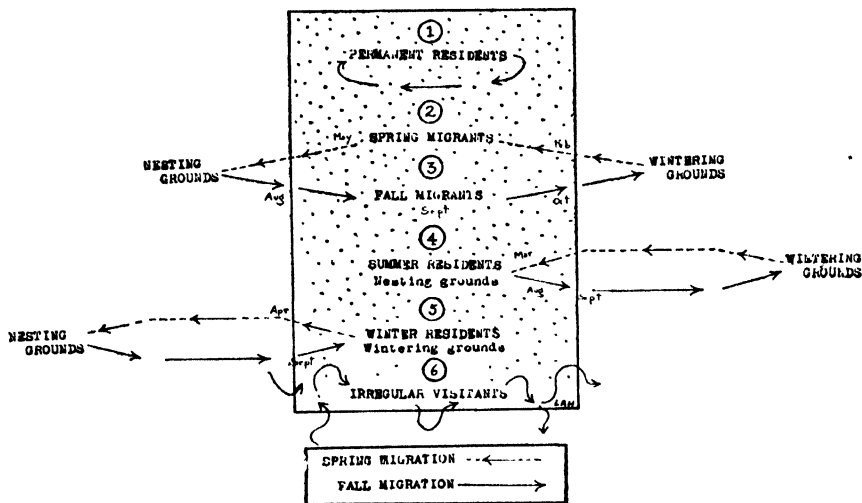


FIG. 1

Chart showing the movements during migration of the six well-defined groups of birds in any given area

For restricted areas, where a careful migration record is being kept, such a chart is very helpful. It can be expanded, and spaces, divided in convenient fashion, left under the name of each group for the listing of the birds of that group, with the dates of their arrivals, departures or nesting.

The plumage and molt chart (Fig. 2) gives the succession of the various plumages and molts for the young and adults of a typical passerine bird. In general these changes among the *Passeres* occur as follows: When hatched the bird is naked, except for a scanty covering of fine down, the first plumage, termed the natal down. The natal down is quickly molted (first molt, postnatal molt) and the second plumage, juvenile plumage, succeeds. Within about two weeks after acquiring this plumage the bird usually is ready to leave the nest, when there occurs the second molt (postjuvenile molt), which leaves the bird with its third plumage (first winter plumage). This in turn is lost by the third molt (prenuptial molt), which takes place in the early spring. In most cases the bird has now acquired its adult plumage with the first nuptial plumage (the fourth since it left the egg), which in the fall by the fourth molt (postnuptial molt) leaves it with its fifth plumage (winter plumage). Thereafter, year by year, it undergoes but two molts, the prenuptial in the spring, and the postnuptial in the fall; and wears but two changes of plumage, the nuptial in the summer, and the winter

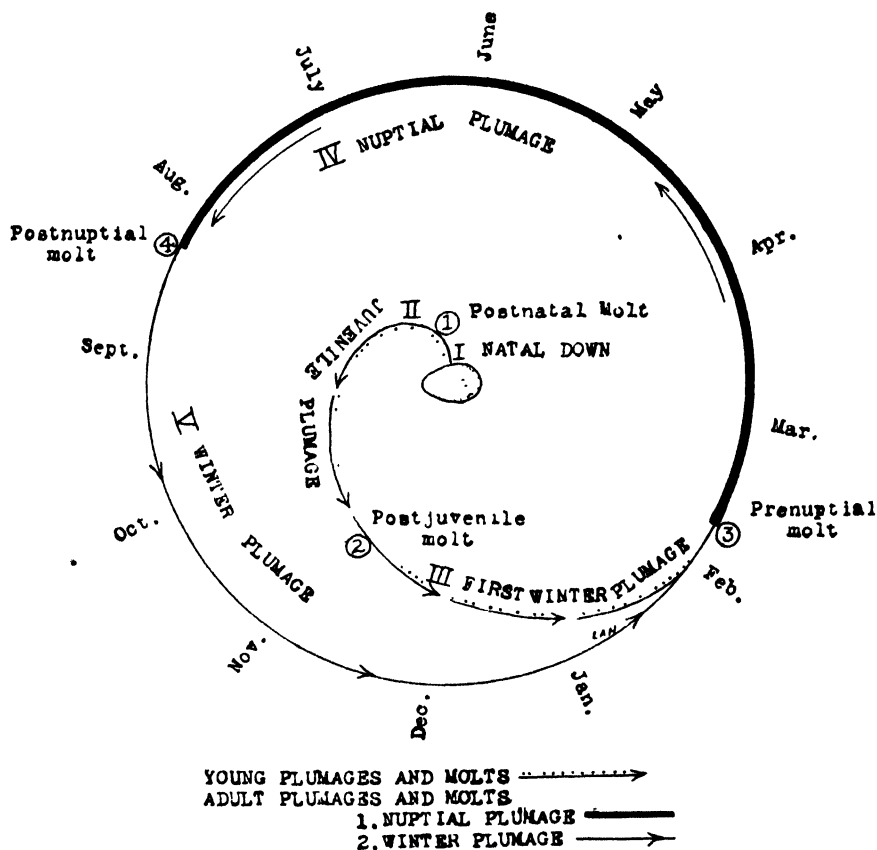


FIG. 2

Chart showing the typical succession of plumages and molts in the young and adults of passerine birds

Plumages in Roman numerals

Molts in Arabic numerals

plumage during the remainder of the year. A multiplicity of specific variations of the above molt and plumage sequence occur among birds, and new plumage colors and patterns are also developed by reason of feather wear, and fading. Such changes are not susceptible of being charted on any general schema, but must be separately recorded for each different species. All birds, however, pass through a complete molt after the breeding season (4, postnuptial molt) after which they acquire the winter plumage. Curiously enough, the males of some birds breed in the immature plumage, as, for example, the orchard oriole (*Icterus spurius*) and the redstart (*Setophaga ruticilla*).

In Fig. 2 the dotted arrows from the egg (center) to the circle

show the succession of immature plumages and molts, and the circle the succession for adults, with the approximate months when the molts occur and the plumages are worn.

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LUTEAL CELLS AND HEN-FEATHERING

WHEN a hen-feathered male is castrated, as shown by Morgan, cock feathers replace the hen feathers. The change is considered by him to be due to the removal of the secretion produced by certain cells, identical with the luteal cells of the ovary, as one of us (N) has recently shown. While N was making histological studies of the developing gonad, the other (G) had become skeptical of the reputed relation between the luteal cells and hen feathering and as a consequence undertook observations and experiments to determine the relationship. Finally, the following test was made. From a large series of sections prepared in G's laboratory at the Massachusetts Agricultural Experiment Station, twelve were sent to N for examination, he being ignorant of the sort of plumage worn by the birds from which the testes were taken. The testes were from young birds in that stage of development where the kind of plumage could be accurately determined if the birds were to become cock-feathered. Only one testis was removed from each bird, and all were allowed to reach maturity. The result of the test is shown in the accompanying table. Nearly all the birds listed are descended from a hen-feathered hybrid male of Morgan's. G is responsible for the data listed in the first two columns, and N for that in the remaining four. His attempt to determine the nature of the feathering from an examination of the sections was based on the condition of the lymphoid cells, for N already had come to doubt the reputed significance of the luteal cells. It is clearly evident, as an inspection of the table shows, that neither luteal cells nor lymphoid cells can be considered causative agents in the production of hen-feathering.

One consideration remains to be stated. In nearly all adult hen-feathered males, the amount of intertubular material is large, contrasted with that found in all cock-feathered males thus far studied by us, but that this material has any causal re-

TABLE I

Band Number	Actual Feathering	Nonidez's Report			
		Feathering	Luteal Cells	Lymphoid Cells	Condition of the Testes
B8218	cock	-----	none	present	juvenile
B8627	cock	hen	none	present	early spermatogenesis
B8628	hen	-----	none	present	juvenile
B8698	cock	hen	present	present	full spermatogenesis
B8734	hen, later changing to cock	-----	none	present	early spermatogenesis
B8896	hen	hen	none	present	early spermatogenesis
B8908	cock	-----	none	present	full spermatogenesis
B9565	hen	-----	none	few	full spermatogenesis
B9567	cock	cock	present	present	full spermatogenesis
B9705	cock	-----	none	few	full spermatogenesis
C2612	hen	hen	present	present	juvenile
C2615	hen	-----	none	present	juvenile

lation to hen-feathering remains to be determined. Such relation seems improbable, and, like the pigment cells in the se-brights' testis, is very likely a mere varietal difference.

H. D. GOODALE
JOSÉ F. NONIDEZ

INTERTUBULAR TISSUE IN THE TESTES OF CERTAIN BIRDS

THE intimate relationship existing between the gonads and plumage in poultry, coupled with the reference of this relationship to the luteal cells, suggested that a study of the gonads of wild birds should be of value. Plans were made for examining most of our common wild birds but illness restricted the examination to a few specimens, collected and sectioned in 1921.

In three species of warblers, *viz.*, chestnut sided, myrtle and Maryland yellowthroat, very large conspicuous cells, considered by Dr. José F. Nonidez, who examined the slides, to be large lymphocytes, form a tissue in the intertubular spaces. Such cells are absent from the testes of a brown thrasher, several English sparrows and domestic ducks, and are found with difficulty in the testes of a robin and a bluebird. These cells are entirely unlike luteal cells, and much larger than the lymphocytes found in the chicken's testis. Consideration of the plumage of these birds makes it evident that no constant relation exists between the cells described and sexual dimorphism of plumage.

Luteal cells could not be identified satisfactorily in the ovary of an adult robin, nor in the juvenile ovaries of the English sparrow and rosebreasted grosbeak. In domestic ducks they are less conspicuous than in domestic chickens.

H. D. GOODALE

THE ABSORPTION OF THE PUBIC SYMPHYSIS OF THE POCKET GOPHER, *GEOMYS BURSARIUS* (SHAW)¹

THE pocket gopher is one of several fossorial animals which has the pelvic girdle greatly reduced, apparently as an adaptation for turning in the narrow confines of its burrow. This reduction has so developed that, in many instances, the pubo-ischiatic-vacuity has become too small for the birth of young and so has necessitated other adaptations. The pubic symphysis of the mole (*Talpidae*) may be absent or, if present, meets dorsal to the digestive and urogenital tracts (Slonaker, 1920)² and the shrews (*Soricidae*) have no symphysis (Lecke, 1884),³ while in the *Rodentia* some of the voles (subfamily *Microtinae*) also are without a symphysis.

From an examination of 56 female pocket gophers, representing 13 species, Chapman (1919)⁴ found that 38 had complete

¹ Contribution No. 68 from the Zoology Department, Agricultural Experiment Station, Kansas State Agricultural College.

² Slonaker, J. R., 1920, Some Morphological Changes for Adaptation in the Mole. *Jour. of Morph.*, Vol. 34, pp. 335-365.

³ Lecke, W., 1884, *Mammalia, pelvis*. Bronn, Klassen und Ordnung des Tierreichs. Bd. 6, s. 571.

⁴ Chapman, R. N., 1919, A Study of the Correlation of the Pelvic Structure and the Habits of Certain Burrowing Mammals. *Amer. Jour. of Anat.*, Vol. 25, pp. 185-208.

pubic symphyses, while in 18 the pubic bones not only did not meet to form a symphysis but were almost absent and spread far apart. He also states that "no intermediate conditions have been found; the bones either meet to form a symphysis or are widely separated. Very young females have been found possessing a symphysis, while old females without a symphysis are not uncommon. It seems, therefore, that the presence or absence of the symphysis can not be a matter of ossification of the bones due to age." In his discussion he further states that "the pelvis is broad and has allowed sufficient room for the passage of the fetuses at the time of birth, and there has, therefore, been no necessity for its loss," and he concludes that "the pocket gophers evidently represent a stage in which the room within the pelvis is greatly restricted and the symphysis is in the process of being lost."

During the last four years the writer has examined over a thousand pocket gophers, *Geomys bursarius* (Shaw), which were collected during all months of the year, and from this material it was found that the development of the pubic bones of both the male and female was identical up to the approach of sexual maturity. The pubic bones are among the last of the pelvis to become ossified and are completely cartilaginous when the animals are two weeks old, while ossification is not complete before they are two thirds grown. During the fall and early winter the ossified symphyses of both full-grown males and females, born during the breeding season of the preceding spring, are complete and similar, but as the next breeding season approaches, when the animals are almost a year old, the pubic bones of the young females begin to be absorbed, first in the symphysis region and then laterally almost to the obturator foramen. All stages in the disappearance of the symphysis have been observed. This absorption is correlated with the general activities of the reproductive system preceding pregnancy and is usually complete before copulation. All of the pregnant females examined had the symphysis completely open, even though the fertilized ova had not implanted, and in no instance has there been any evidence to indicate that the symphysis is restored after parturition. Careful measurements of newly born young show that they could not pass through the pelvic opening if the pubic symphyses were present. These facts seem to indicate that the absorption of the pubic symphysis of the female pocket gopher is an adaptation to compensate for a reduction of

the pelvic cavity which is correlated with the animal's fossorial habits.

Of the 500 adult females examined only three having a complete symphysis were taken during the breeding season, and in these cases one had deformed reproductive organs and those of the other two were in a non-active, juvenile condition. It has also been found that young females having cartilaginous pubes when placed in captivity will develop bony symphyses like those of gophers in the field, but it is difficult to regulate laboratory conditions so that the symphyses will be absorbed. Some pocket gophers have been kept in captivity for over eighteen months without any apparent change in the symphyses. These captive females grow to full size and appear normal in every respect, but an examination has always revealed that in addition to a closed symphyses the reproductive system was in a juvenile and non-active condition. The two horns of the uterus were never found congested and appeared as white bands, while the ovaries were small and smooth, showing no signs of follicular activity.

The pubis of the male pocket gopher is different from that of the female in that it persists throughout life. In an examination of over 500 males one had a small notch separating the two pubic bones. These observations on the males agree with those of Chapman ('19), who states that all of the male specimens he examined had the pubic bones uniting to form a symphysis.

The fact that the female pocket gophers lose their symphyses during the breeding season and that the loss is always associated with active ovaries and uteri seems to indicate that the real causative agent is supplied by some part of the reproductive system. It is also significant to note that this seems to be peculiar to the female reproductive system because, even though the male reproductive organs undergo very marked changes during the breeding period, the pubis is not absorbed. Experiments are in progress to determine the part of the female reproductive system to which the absorption of the symphysis is due, and the data already obtained seem to indicate that the ovaries supply a factor.

It has been found that castrated males lose their symphyses if given intraperitoneal injections of ovarian extracts. Also the time intervening between castration and the administering of the extracts seems to have a direct bearing upon the length of time and number of injections required to cause the male to lose the symphysis (Table I). Males that have been castrated four

TABLE I

SHOWING THE INFLUENCE OF OVARIAN EXTRACT ON THE PUBIC SYMPHYSES OF CASTRATED MALE POCKET GOPHERS

No. of Animal	Date castrated	Date of 1st injection	Roughenings of pubic bones	Notch at symphysis	No. of injections	Time in days from 1st injection
113	6- 5-22	11-17-22	?	1- 1-23	6	45
114	4-19-22	11-17-22	12-17-22	1- 6-23	8	50
144	12- 1-22	12- 7-22	1-15-22	2-19-23	11	74
153	11-29-22	12- 2-22	2- 1-23	2-19-23	11	79
164	11-12-23	11-10-22	3- 5-23	3-19-23	17	129

to seven months (Nos. 113 and 114) before treatment usually lose their symphyses in six to eight weeks when injected intraperitoneally once a week with three cubic centimeters of normal saline solution containing about one grain of desiccated ovarian substance of the sow, while males that are castrated and receive this treatment immediately (Nos. 114, 153 and 164) require from eleven to seventeen weeks to lose the symphysis. It has also been found that adult females having a pubic symphysis lose it if given ovarian extracts, as described for the male, and the time and number of treatments required is about the same as that for males that have been castrated for several months. In both males and females the first indication that the symphysis is being absorbed is a roughening of the pubic bones. This roughening is easily detected by palpation and occurs two to four weeks before a median notch separating the pubic bones is formed at the symphysis.

Work is in progress to determine the effect of ovarian extracts on the symphyses of uncastrated males, both during the breeding season when the testicles are large and secreting spermatogenic materials, and out of the breeding season when they are small and do not contain functional spermatozoa. Studies are also being made of the effects of ovarian extracts on the cartilaginous symphyses of young males and females to see if it is possible to cause absorption of the cartilage before it is ossified. The possibility that the ovarian extract has a definite relationship to the calcium balance is also being taken into consideration and a study is being made of the calcium metabolism of both normal and experimental animals.

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THE VALUE OF THE STUDY OF MITOCHONDRIA IN CELLULAR PATHOLOGY¹

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THE problem is a wide one, because it has been shown conclusively in the past few years that mitochondria are almost though not quite coextensive in distribution with living protoplasm. They have attracted the attention of botanists, zoologists, anatomists, physiologists, pathologists and clinicians, who have all studied them from varied points of view. A conservative estimate would place the bibliography of the last decade at about 600 papers, scattered widely in the journals of all countries.² Any account of the results obtained and the outlook for further investigation is bound to be somewhat narrow and one-sided. A botanist might emphasize the mitochondrial origin of plastids, base thereon the thesis that without mitochondria it is questionable whether we could have any green plants, and go on to philosophise about all life being dependent upon mitochondria. A zoologist would, in all likelihood, devote considerable time to a discussion of the behavior of mitochondria in the developing egg and sperm, their rôle in fertilization and histogenesis, and the possibility that they may be concerned in the hereditary transmission of certain characteristics.

¹ Annual Samuel G. Gross Lecture of the Philadelphia Pathological Society, 1923.

² Reviews: Duesberg, J., *Ergebn. d. Anat. u. Entwicklungs-gesch.*, 20, 567-916, 1912. Benda, C., *Verhandl. d. deutsch. path. Gesellsch.*, 5-42, 1914. Cowdry, E. V., *Section on Mitochondria in a Text-book of General Cytology* to be published by the University of Chicago Press, April, 1924.

A physiologist would stress the undoubted but obscure relation of mitochondria to cellular activity. Each specialist would certainly have his own distinctive reaction, so that the best that I can hope to do is hurriedly and incompletely to review the subject as it appeals to an anatomist keenly interested in pathology. As a background for discussion it may be helpful to mention briefly certain properties of mitochondria which have been definitely established.

(1) *Occurrence.* In bacteria it is very questionable whether mitochondria exist, although they have been described by Alexeieff³ and granules may be observed in some of the larger forms which stain more or less specifically with janus green. They have, however, been repeatedly found in the algae and fungi and in a very primitive group called mycetozoa, since its members partake of the properties of both plants and animals. Thus far they have been reported with but few exceptions in all the higher forms of both kingdoms which have been adequately studied. In their breadth of distribution they rival the nucleus. They are present in embryos, in the young, in adults and in the aged. But it is recognized that in the terminal stages of cytomorphosis, when the cells are actually moribund, they tend to disappear, as, for instance, in non-nucleated red blood cells and desquamating epidermal cells. It is worthy of note that in the life cycle of erythrocytes they persist for an appreciable time after the loss of the nuclei. An interesting parallel may be drawn between the behavior of mitochondria during the formation of hemoglobin in animals and the development of chlorophyll in plants. In both cases, with the aging of the cell, there is a disappearance of mitochondria and the formation of pigment.

Now that the first flush and excitement of discovery are past, we may, in the natural course of events, expect a little retrenchment from unqualified statements to the effect that mitochondria occur in *all* cells. In other

³ Alexeieff, A., C. rend. Soc. de Biol., 89, 728-730, 1923.

words, it is probable that lowly plants and animals—and also certain tissue cells of higher forms—will soon be reported in which mitochondria do not occur or in which the materials said to represent them differ so widely from the typical mitochondria of vertebrates that the term can not rightly be applied to them. For example, among the protozoa, the paraplasmids pass through a stage of development within the red blood corpuscles of vertebrates in which the cytoplasm is very much reduced, and *anaplasma marginale* (the parasite of East Coast Fever) is said during this phase to consist wholly of nuclear material.⁴ It would be a crucial test to ascertain whether with an increase of cytoplasm mitochondria appear, because, if so, it would be a clear case of their *de novo* origin.

(2) *Morphology.* Throughout the length and breadth of the world of living things, wherever they occur, the morphology of mitochondria is remarkably uniform. Comparing again the two great divisions of nature, we find a similar range of variation in the shape of mitochondria in animals and plants.⁵ Indeed, it would be a difficult task to find any other cellular component which is so nearly identical in the two. Passing to mammalian and human tissues, which interest us more directly, we observe that different cells are fitted to perform their duties with mitochondria of more or less distinctive shape. Thus, a marked difference is noticeable in the various categories of human blood cells. They tend to be unusually filamentous in gland cells, in nerve cells and in tissues of the developing embryos of all vertebrates. Although their length varies from say 0.5 to 10 microns and their diameter from about 0.5 to 1 micron, their girth in the cells of the same tissue is astonishingly uniform. From this we assume that interactions between the cytoplasm and the mitochondria can only profitably

⁴ Theiler, Sir Arnold, 1910. "Texasfieber, Rotwasser und Gallenkrankheit der Rinder." *Zeitschr. f. Infektionskrankheiten der Haustiere*, 8, 39; Velu, H., 1922. "Les piroplasmes et les piroplasmoses." Paris, E. Larose, 285 pp.

⁵ Cowdry, N. H., *Biol. Bull.*, 33, 196-228, 1917.

take place in a certain thickness of mitochondrial substance, so that we have two attributes—length and breadth—independently variable and probably influenced by different factors.

(3) *Arrangement.* Within individual cells, mitochondria are usually distributed without definite order throughout the cytoplasm, but some interesting exceptions are to be noted. In the parotid they are clumped in the proximal pole; in intestinal epithelial cells at both poles; and in the thyroid of the opossum, according to Bensley,⁶ in the distal cytoplasm. So that there is some reason to believe that they may act as indicators of secretory polarity, like the Golgi apparatus.

Perinuclear condensations of mitochondria have been observed repeatedly in both plants and animals. To illustrate, we may refer to the early meristem in which no definite arrangement is noticeable while in the older cells mitochondria come into actual contact with the nucleus, where they enlarge to form plastids, which finally migrate away from the nucleus and become more or less evenly distributed in the surrounding cytoplasm. This migration has been noted repeatedly by botanists who have detected a coincident increase in their resistance to the solvent action of acetic acid. Similarly, in the spermatogonia of certain animals the mitochondria make their way to the nucleus and become so closely applied to it that some investigators have been led to think that they actually originate from it. In the later stages of spermatogenesis they leave the vicinity of the nucleus and become more resistant to acetic acid. Actual movements to and fro have been observed by W. H. and M. R. Lewis⁷ in living tissue cultures.

It is also a common experience to find mitochondria gathered in the peripheral cytoplasm, especially in egg cells. In cell division mitochondria are distributed approximately equally to the daughter cells. They are often radially disposed about the centrosome. Numerous other

⁶ Bensley, R. R., *Am. J. Anat.*, 19, 37-54, 1916.

⁷ Lewis, M. R., and W. H., *Am. J. Anat.*, 17, 339-401, 1915.

modifications have been noted, dependent upon variations in pressure and other obvious causes, but not a shred of evidence has been found that mitochondria possess powers of independent motility like some bacteria, which would explain the changes in position.

(4) *Amount*. In young embryos most of the cells contain approximately the same number of mitochondria per unit volume of cytoplasm. As development advances distinctive differences in the amount of mitochondria arise with the specialization of tissues. They are sometimes relatively more abundant soon after birth than in adults, notably in the pancreas and the thyroid. It has already been mentioned that they disappear as the cells become senile. To estimate these alterations quantitatively is particularly difficult in gland cells on account of the cyclical changes in volume, but a beginning has been made in nerve cells by Thurlow⁸ who worked out a method of counting them in unit areas. She found marked differences in the relative amount of mitochondria in the cells of certain cranial nerves, thus contributing a line of advance which should be extended to pathological conditions.

(5) *Chemical constitution*. The suggestion that mitochondria are phospholipins in protein combination has come from three chief sources: From Regaud's⁹ study of mammalian tissues, from Fauré-Fremiet's¹⁰ work on protozoa, and from the investigations of the botanist Löw-schin¹¹ which have unfortunately not been confirmed. In character it is largely negative. They do not color with Sudan III nor exhibit any of the properties of polysaccharides. Neither do they apparently contain appreciable amounts of iron in protein combination (Macallum's test). Berg,¹² Noël¹³ and others have applied Millon's

⁸ Thurlow, M. DeG., *Contrib. Embryol. (Carnegie Inst.)*, Washington, 6 (16), 35-44, 1917.

⁹ Regaud, Cl., *Compt. rend. Soc. de Biol.*, 65, 718-720, 1908.

¹⁰ Fauré-Fremiet, E., *Arch. d'Anat. micr.*, 11, 457-648, 1910.

¹¹ Löw-schin, A. M., *Ber. d. deutsch. bot. Gesellsch.*, 31, 203-209, 1913; 32, 266-270, 1914.

¹² Berg, W., *Arch. f. mikr. Anat.*, 96, 54-76, 1922.

¹³ Noël, R., *Arch. d'Anat. micr.*, 19, 1-158, 1923.

reagent quite extensively to liver cells without obtaining a pronounced coloration of mitochondria. But mitochondria do occasionally blacken with osmic acid. They are soluble in alcohol, ether, chloroform and other similar reagents unless they have been rendered relatively insoluble by fixation. Preservatives containing acetic acid in a concentration of from 5 to 10 per cent. usually destroy them. In appropriately fixed tissues, they are strongly fuchsinophile and stain sharply with iron hematoxylin. Perhaps their most striking characteristic is their affinity in living cells for Janus green B, which will color them in a dilution as great as one in half a million of physiological salt solution. If, however, H_2 or $(CH_3)_2$ is substituted for the $(C_2H_5)_2$ group in the compound, the specificity is lost.¹⁴

When we speak of homology between the mitochondria of different cells, we can only do so tentatively and with due qualification because, upon the discovery of more exact methods, further differences in composition may be brought to light. In other words, we have before us material exhibiting certain general properties in all living and active cells but which nevertheless varies to some extent in its attributes just as chromatin does.

(6) *Nature*. So close is, in some cases, the morphological resemblance between mitochondria and bacteria that investigators have from time to time asserted that they are in truth living microorganisms. This was the opinion of Altmann¹⁵ who deserves credit for the prominent part which he took in bringing them to our attention (1880-1890). But his colleagues in Leipzig and in other parts of Germany did not receive his suggestion with enthusiasm, partly because he conceived the ground substance to be a lifeless substratum. Several years later, Portier¹⁶ strongly advocated a somewhat similar view

¹⁴ Cowdry, E. V., *Contrib. Embryol. (Carnegie Inst.)*, Washington, 8 (25), 39-160, 1918.

¹⁵ Altmann, R., "Die Elementarorganismen und ihre Beziehungen zu den Zellen." Leipzig, Veit and Co., 145 pp., 1890.

¹⁶ Portier, P., *Compt. rend. Acad. d. sc.*, 165, 267-269, 1917.

"Les Symbiotes," Paris, Masson and Cie, 315 pp., 1918.

Compt. rend. Soc. de Biol., 82, 247-250, 1919.

that mitochondria are symbiotic bacteria but his contention has not been favorably received¹⁷ and the same idea advanced independently in the United States by Wallin¹⁸ has also been questioned.¹⁹ The generalization proposed is indeed so far-reaching in its application that the need for clear-cut information has been emphasized in a recent editorial published in the *Journal* of the American Medical Association.²⁰ The salient point of the discussion seems to be the manner of interpretation of the differences which undoubtedly exist between mitochondria and bacteria. Wallin is of the opinion "that mitochondria are symbiotic bacteria in the cytoplasm of all higher organisms whose symbiotic existence had its inception at the dawn of phylogenetic evolution." It is natural that during millions of years their properties would become somewhat changed from free living bacteria and he is inclined to explain some of the differences on this basis. Whether all differences are of a kind which may reasonably be expected to arise in this way I seriously question. Neither can I accept his contention that other organisms which have enjoyed a symbiotic relationship for a relatively short time approach mitochondria in their properties to a degree which would lend any support to his theory. This he has claimed for the *Bacillus radicolica* and the parasite of Rocky Mountain spotted fever as described by Wolbach, but very recently these organisms have been clearly differentiated from the mitochondria lying side by side in the same cell.²¹ Not

¹⁷ Regaud, Cl., *Compt. rend. Soc. de Biol.*, 82, 244-247, 1919.

Laguesse, E., *Ibid.*, 337-339.

Guilliermond, A., *Ibid.*, 309-312.

Rasmussen, A. T., *J. Comp. Neurol.*, 31, 37-49, 1919.

Van Gehuchten, P., *Compt. rend. Soc. de Biol.*, 84, 652-654, 1921.

Caullery, M., "Le Parasitisme et la Symbiose," Doin, Paris, 1922.

Levi, G., *Monit. Zool. Ital.*, 33, 99-118, 1922.

¹⁸ Wallin, I. E., *Am. J. Anat.*, 30, 203-229, 451-467, 1922. *Anat. Record*, 25, 154, 159, 1923. *AM. NATURALIST*, 57, 225-261, 1923.

¹⁹ Cowdry, E. V., and Olitsky, Peter K., *J. Exper. Med.*, 36, 521-533, 1922.

²⁰ *J. Amer. Med. Assoc.*, 79, 1848, 1922.

²¹ Cowdry, E. V., *Am. J. Anat.*, 31, 339-345, 1923.

Nicholson, F. M., *J. Exper. Med.*, 37, 221-230, 1923.

only is there a very great gap between the properties of bacteria which have developed the most perfect degree of symbiosis known to us and mitochondria, but the positive data which has accumulated regarding the latter does not readily lend itself to interpretation in terms of this hypothesis.

A wholly different idea has dominated most of the work on mitochondria, namely, that during development they are transformed into many products of cellular differentiation,²² a conception which falls well in line with the view that they are in part the material basis of heredity. A list of 80 substances, in the formation of which they are said to be concerned, was published in 1918. Many others may now be added. They comprise materials of the most diverse character, including glandular secretions, pigments, leucocytic granules, plant plastids, fibrillar structures of different kinds, fat, protein, glycogen, starch, urea, etc. That many of them are laid down either within the mitochondria or in their immediate vicinity is an observation of far-reaching importance which can not reasonably be questioned—the evidence contributed by Guilliermond through the direct observation of living plant cells is particularly convincing—but the likelihood that an actual transformation of mitochondria takes place naturally depends upon the difference in the chemical constitution of the original substance and the supposed end products, so that some of the claims involve chemical and physical improbabilities. The word transformation is certainly too definite and exclusive.

It was perhaps to overcome this objection that Regaud²³ advanced his famous ectosomes theory according to which the mitochondria play the part of plastids (into which some of them are known to develop in plants), choosing and selecting substances from the surrounding cytoplasm, condensing them and transforming them in their interior into infinitely diverse products.

²² Meves, F., *Arch. f. mikr. Anat.*, 72, 816-867, 1908; *ibid.*, 92 (2), 41-136, 1918.

²³ Regaud, Cl., *Rev. de Med.*, 31, 681-699, 1911.

He compared them to the side-chains of Ehrlich. His theory is essentially a modification of the lipoid membrane conception of Overton, with this difference, that the lipoid is thought to be scattered throughout the cytoplasm, in the form of mitochondria, instead of being restricted to a layer upon the surface of the cell.

However this may be, to take part in the formation of certain products of differentiation is certainly not the only function of mitochondria, because we have already remarked upon their universal occurrence in embryonic cells before the onset of specialized activities. Impressed by their wide distribution, investigators have felt obliged to entertain the view that they are also concerned in some very fundamental vital phenomenon. Many have suggested metabolism; a few others, respiration; further study alone can decide.

(7) *Changes in pathological conditions.* Since mitochondria differ radically from the nucleus, the hope has been frequently expressed that their study will open an entirely new chapter in cellular pathology.²⁴ This expectation seems likely to be fulfilled because they are purely cytoplasmic elements which it is reasonable to suppose will be much more directly concerned than the nucleus in the manifold adjustments which are constantly taking place between living cells and their environment. It is not surprising, therefore, that mitochondria have been somewhat hastily studied in a great variety of pathological conditions including acute and chronic infectious diseases, endocrine disturbances, tumors, different dietary states, injury of mechanical or chemical nature, etc., etc.

In the literature, a complication is added by the fact that mitochondria have come to the attention of investigators in many branches of the biological and medical sciences who have shown no hesitancy in the invention of new terms to indicate theoretical interpretations, morphological characteristics, physical consistency and a host of other observed and supposed properties, so that in practice we have to seek information under a variety

²⁴ See editorial J. Amer. Med. Assoc., 67, 813, 1916.

of headings of which the following are most important:²⁵ chondriokonten, chondriosomen, chondriorhäbden, chondrioplasten, chondriosphären, chromochondria, karyochondria, myochondria, plastosomen, plastochondrien, vermicules, perinème, mitogels, mitosols, and fuchsino-phile and interstitial granules (in part).

At this stage it requires some knowledge and no little imagination to select any type of injury in which the mitochondrial changes have not already been touched upon, usually by the examination of human tissues taken at operation, or at autopsy in which adequate control is out of the question, for the reason that the conditions can never be exactly duplicated. Failure to recognize the fact that a delicate indicator of cellular injury, like a new chemical reagent, is likely to be misleading is responsible for some disappointment in the results obtained. However precise the instructions may be the technique can not properly be shifted on to the shoulders of a technician. The methods require some adjustment to each tissue and must be standardized by frequent repetition. Even a slight and apparently trivial deviation from routine will often produce unexpected and profound alterations in the mitochondria. Pinching the tissues with the forceps or letting their surfaces dry in air will render them useless for accurate work. Much has been written about the necessity of taking them from the body immediately after death. This depends, naturally, upon the rate of autolysis. It is essential with glands and less so with the nervous system.

For many years the study of mitochondria in pathology will demand the painstaking observation of their behavior in animals held under experimental conditions which are capable of rigid control. We have to work over in detail ground which has been hastily scanned by our predecessors. But in this slow and systematic review, a good beginning has already been made.

²⁵ Cowdry, E. V., *Anat. Record*, 22, 239-250, 1921.

The term "mitochondria" (thread granules) is derived from the Greek *μήτρος*, a thread, and *χόνδρος*, a grain.

It has been shown, for example, that mitochondria are very much more sensitive in some tissues than in others. In glands they often respond by a loss of filamentous shape a considerable time before the nuclei exhibit any noticeable modifications. This has been found in experimental phosphorus poisoning by Scott,²⁶ who made the further observation that they are concerned in the resultant fatty degeneration. Nicholson²⁷ has brought to light a variety of mitochondrial changes in the thyroid which, as in the case of phosphorus poisoning, would probably have been completely overlooked in ordinary preparations fixed in Zenker's fluid and stained with hematoxylin and eosin. Other instances of the extreme susceptibility of mitochondria in gland cells might be cited from the almost overwhelming literature.

It is quite otherwise with the nervous system in which mitochondria respond to serious injury like axone section,²⁸ but show no very characteristic changes in beriberi,²⁹ functional exhaustion,³⁰ hibernation,³¹ poliomyelitis,³² and experimental herpetic encephalitis.³³ No explanation has been vouchsafed to explain this variability in reactivity, but it may be a function of the properties of the cytoplasm in which the mitochondria are imbedded and may not signify any radical differences in the mitochondria themselves.

Three general modes of mitochondrial reaction are recognized—qualitative, quantitative and topographical—which may occur singly or in combination.

By far the most delicate qualitative response is a change of filamentous mitochondria into granules. It is so delicate that it is often produced through injury caused by faulty technique alone. We have already men-

²⁶ Scott, W. J. M., *Am. J. Anat.*, **20**, 237-253, 1916.

²⁷ Nicholson, F. M., *J. Exper. Med.*, 1924.

²⁸ Luna, E., *Anat. Anz.*, **44**, 413-415, 1913.

²⁹ Clark, E., *J. Comp. Neurol.*, **24**, 61-110, 1914.

³⁰ Strongman, B. T., *Anat. Record*, **12**, 167-171, 1917.

³¹ Rasmussen, A. T., *J. Comp. Neurol.*, **31**, 37-49, 1919.

³² McCann, G. F., *J. Exper. Med.*, **27**, 31-36, 1918.

³³ Cowdry, E. V., and Nicholson, F. M., *J. Exper. Med.*, **38**, 695-706, 1923.

tioned its occurrence in the pancreas in phosphorus poisoning, and in the thyroid under a variety of conditions. It is common in inanition³⁴ and has been repeatedly observed in the living cells of tissue cultures.⁷ Evidently cytoplasmic conditions favorable to a filamentous type of mitochondria may be upset or disturbed in a variety of ways, so that the change to granules can not be regarded as in any sense specific. We can not tell whether the injury acts directly upon the mitochondria or whether the morphological changes are only the visible expression of a long line of interdependent chemical reactions. Occasionally an increase in the girth of mitochondrial filaments is to be noted, but very rarely are they observed to elongate as a result of injury. Quite frequently they swell up into droplets with pronounced fatty and lipoidal properties. These alterations in form will probably remain obscure until we are able to correlate them with chemical changes by the aid of more exact methods for the detection of the lipoidal and protein fractions which probably enter into the composition of mitochondria. Changes in the shape of mitochondria produced in living cells outside the body by the action of hypo- and hypertonic solutions, by acids and bases, and by alterations in temperature are interesting and significant, but the limited range of variation in these qualities of the circulating blood precludes their operation except in a minor degree and we are left to explain why mitochondria existing side by side in the same cell, and presumably sharing most influences of this kind in common, often differ so greatly in morphology.

Quantitative changes are equally likely to be misleading. Even with uniformly fixed tissues unless the stain is differentiated to exactly the same extent an illusory impression of decrease or increase in mitochondria may easily be created. With rare exceptions, the observations recorded in the literature are merely based upon the gen-

³⁴ Miller, S. P., *Anat. Record*, 23, 45, 205-210, 1922. Okuneff, N., *Arch. f. mikr. Anat.*, 97, 187-203, 1923. Ma. W. C., *Anat. Record*, 25, 157, 1923.

eral appearance of sections. But few investigators have availed themselves of Thurlow's⁸ method of counting the mitochondria in unit areas³¹ and have taken the trouble to measure the variation in the size of the cells.¹³ A diminution in the number of mitochondria is often met with in pathological conditions, but an increase above normal is rare. It has, nevertheless, been reported in toxic adenomata of the thyroid,³⁵ in the islets of Langerhans of the pancreas during diabetes,³⁶ in the kidney following the administration of phloridzin,³⁷ in regeneration³⁸ and, under the heading of Altmann's granules, in compensatory hypertrophy.³⁹ In tumors great variability in the number of mitochondria has been noted.⁴⁰ It is, I think, safe to assume that a decrease is to be considered a sign of depression of functional activity and that an increase is indicative of heightened activity, provided that they retain their normal shape, but when the increase is manifested by a swelling up of mitochondria into rounded droplets of different sizes, the condition is apt to pass insensibly into a simple accumulation of fat and lipoid which would point, on the other hand, to a decrease in the rate of intracellular oxidation. For the present, even a conspicuous increase in filamentous and rod-like mitochondria or of tiny granules hardly justifies the conclusion that any specific type of physiological process is enhanced, but it does show that the cells are not in a dormant or inactive state.

³⁵ Goetsch, E., Johns Hopkins Hosp. Bull., 27, 129-133, 1916.

³⁶ Homans, J., J. Med. Research, 33, 1-51, 1915.

³⁷ Policard, A., Arch. d'Anat. micr., 12, 177-288, 1910.

³⁸ Romeis, B., Anat. Anz., 45, 1-19, 1913.

Torraca, L., Arch. f. Zellforsch., 12, 539-552, 1914. Anat. Anz., 45, 459-474. Arch. ital. di anat. e di embriol., 15, 283-330, 1916.

³⁹ Enderlen, Deut. Zeitschr. f. Chir., 41, 208, 1908.

Hirsch, C., Anat. Hefte, 41 (1), 131-172, 1910.

De Giacomo, H., Intern. Monatschr. f. Anat. u. Phys., 28, 208-232, 1911.

⁴⁰ Veratti, E., Boll. Soc. Med. Chir. di Pavia, 23, 34-45, 1909.

Beckton, H., Arch. Middlesex Hosp., 15, 182-191, 1909.

Bensley, R. R., Trans. Chicago Path. Soc., 8, 78-83, 1910.

Favre, M., and Regaud, Cl., Compt. rend. Soc. de Biol., 74, 608-611, 1913.

Porcelli-Titone, F., Beitr. z. path. Anat. u. z. allg. Path., 58, 237-249, 1914.

Sokoloff, B., Compt. rend. Soc. de Biol., 87, 1202-1204, 1922.

By contrast, changes in the position of mitochondria within the cell are much less likely to result from imperfect technique. They have been reported in several conditions. The peripheral margination, discovered by Grynfeldt and Lafont,⁴¹ is particularly noteworthy. A grouping of mitochondria about the nucleus is often met with in tumor cells and is generally, but not always, associated with a change in form. Here also we are at a loss to suggest any rational explanation and shall have to await an experimental analysis upon a fairly comprehensive scale of conditions which are associated with migration back and forth.

But mitochondria may possibly be of value in cellular pathology not only as indicators but in other ways. Recognition of the fact that they occur in the vast majority of living cells opens up a new line of study in the reexamination of the genesis of cytoplasmic inclusions of doubtful nature described before their discovery. For example, the large group of exanthematic diseases of unknown causation, including vaccinia, small pox, herpes, chicken pox and several others, that are less familiar, offer many remarkable cytoplasmic inclusions, concerning which more information is urgently needed.

(8) *Outlook for further study.* Unfortunately, the positive achievements of the study of mitochondria are as yet somewhat intangible. They constitute more than anything else a good augury for the future. However, when finally we attempt to take stock of the situation, several points become clear to us.

In the first place, it is quite obvious that the investigation of mitochondria will never achieve the usefulness which it deserves as an instrument for advance in biology and medicine until we know much more of their chemical constitution as the only accurate basis for interpretation of our findings. In other words, we must wait

⁴¹ Grynfeldt, E., and Lafont, R., Montpel. med., 43, 495, 502, 1921. Compt. rend. Soc. de Biol., 85, 292-293, 406-408, 1921. Compt. rend. Acad. d. sci., 173, 257-260, 1921.

upon the slow development of direct qualitative cellular chemistry.

Secondly, we have to admit that we are still, more than thirty years after the discovery of mitochondria, working very much in the dark. It is not greatly to our credit that these elements, which we can all clearly see at will in living cells, merely by the addition of a drop of janus green and the use of a good microscope, should continue to be such a profound mystery. Neither is it gratifying to find how relatively insignificant has been our progress in the last five or six years. We have a plethora of observations, but no new experimental method has brought us noticeably nearer to a solution of the puzzle.

We naturally search for new avenues of approach, and it is difficult to suggest any, but it is possible that we may use to better advantage methods already at hand. Even in experimental animals the physiologic processes are of such complexity as to be very baffling. To simplify matters we may have to resort to the systematic examination of pure lines of cells in tissue cultures by methods like those recently elaborated in a series of contributions by Carrel and Ebeling⁴² which make possible quantitative study and the accurate analysis of cellular behavior in media of known chemical constitution. We may also profit by the very considerable advances made by W. H. Lewis and M. R. Lewis,⁷ Levi,⁴³ and other cytologists with saline solutions as media. But further improvements are required even in a technique which achieves results that a generation ago would have seemed almost miraculous. It is desirable for one thing to approach still more closely to conditions in the living organism. The basis for ideal experimentation involves a method whereby living cells may be kept in good condition without evincing changes of a degenerative nature over a fairly long period of time, and by which cells of the same type show an equally uniform mitochondrial picture as in the living animal. This can only be accomplished by a

⁴² Carrel, Alexis, and Ebeling, A. H., *J. Exper. Med.*, *37*, 759-766, 1923.

⁴³ Levi, G., *R. Accad. d. Lincei*, *31*, 425-428, 1922, and many earlier papers.

kind of artificial circulation providing for continual replenishing of the culture medium. Under the best of conditions interpretation will be difficult. It will not be possible, for instance, to say that the mitochondria, or the cells containing them, behave in exactly the same way as they do in the organism *in toto*. Since the cells find themselves unrestrained by the regulative processes to which they are accustomed, we may look for somewhat exaggerated reactions which take place at rates different from the normal. But with this limitation, advance along these lines may be instrumental in elevating the study of the cell to a truly experimental science dealing with variables that can be accurately measured and controlled.

There is still another point that I would emphasize. It is possible that students of mitochondria have approached too close to the problem to see it in its proper proportions. The tendency has been to single out the mitochondria and to observe closely their behavior under many conditions. The analogy is not very close, but the concentration of all our faculties upon the mainspring of a watch would not tell us very much unless its behavior were considered in relation to all the other parts of the mechanism. It is a sign of the times that more and more emphasis is being placed upon the interdependence of vital processes. Thus, Osterhout⁴⁴ is of the opinion "that life depends upon a series of reactions which normally proceed at rates which bear a definite relation to each other"—and "that a disturbance of these rate-relations may have a profound effect upon the organism, and may produce such diversive phenomena as stimulation, development, injury and death." Certainly, the most wonderful attribute of a living cell is the harmony of the chemical processes taking place within it. May we not profitably shift our method of approach and strive to modify cellular activity as nearly as possible in one direction only, and if possible to a degree that can be quan-

⁴⁴ Osterhout, W. J. V., The Harvey Lectures, New York, Lippincott, 1921-22, pp. 174-178.

titatively measured and then proceed to an intensive examination of the inter-relationships of the resultant structural and chemical changes? Correlations might well be effected between the composition of the medium in which the cells are living, some physiological attribute which can be measured and reduced to mathematical terms like growth, the size and internal morphology of the cells and their microchemistry. What might also be very helpful would be the direct observation of the physical condition of the cells by the methods of Kite and Chambers, who are responsible for the perfection of an apparatus for microdissection based upon Barber's pipette holder. The more skilful the analysis in selecting likely variables, and the more efficient the necessary organization, the better would be our chance to unearth phenomena causally related and thus to obtain an inkling of the method of integration of vital processes within the cell and of the part played therein by mitochondria.

Since the days of Schleiden and Schwann, cytology has been almost wholly an individualistic study. There have been no instances of the development of organization and cooperation at all comparable to that considered necessary in medicine and other applied sciences. As a rule, the cytologist attacks the most difficult and tricky of all problems—the nature of vital processes within the cell—armed only with a good microscope, a few chemicals, and an abundance of enthusiasm. That a more comprehensive and systematic approach will bear fruit is shown clearly by the work of Morgan and his associates in their experimental studies on the chromosomes.

Without dogmatism it may be said that our point of view in respect to mitochondria falls directly in line with the attention which is now being directed to physical forces acting at surfaces of separation between fluids of different character and density. Certainly one of the most fundamental features of cellular architecture is the presence of almost innumerable mitochondria which in the aggregate afford a surface far greater in extent than

that of the nuclear or plasma membranes. While fibrils, secretion granules, centrosomes and all other known products of protoplasmic differentiation are but short-lived and even the nuclear membrane is (during cell division) periodically lost and reconstituted, the mitochondria-cytoplasmic complex, alone, is inseparable from phenomena which we call vital and has so endured for years without number. The topographic association in the cell between mitochondria and chemical changes of great variety I have already mentioned. This is, happily, not a theory but a fact established by direct and repeated observation.

In concluding, it is not my intention in any sense to leave the impression that we are upon the threshold of the solution of this problem or upon the brink of any important discovery regarding the cell. However we proceed, many years of patient endeavor are required during which each of us will continue to use the methods which he is best qualified to handle. Inasmuch as we only dimly appreciate the meaning in cellular physiology and pathology of the more familiar surfaces of separation, we must steel ourselves to many disappointments before, with good fortune, we even approximate to a correct interpretation of mitochondria.

THE PROBLEM OF PATTERN IN ORGANISMS¹

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I. GENERAL SURVEY

LIVING organisms are more or less distinctly defined and delimited individuals. An individual organism is an entity which possesses an order or unity of some sort which distinguishes it from its surroundings. Each organism possesses in some degree both morphological and physiological order and unity, and the problem of pattern is the problem of the basis and nature of this order and unity. Pattern, as the term is used here, includes not only the gross morphological and developmental pattern, but also physiological and physico-chemical pattern, that is, the pattern of the chemical reactions, the energy-transformations and transfers, the colloids and their changes, and so on. In fact, the problem of pattern in organisms in its broadest sense is the question, What is the organism? While we are still far from a complete answer to this question, it appears in the shifting light of biological research now in one aspect, now in another, and every advance in knowledge makes necessary a new consideration of it. The following discussion is concerned primarily with certain physiological aspects of the problem and with the bearing upon it of certain lines of recent investigation. The conception of the unity and order, that is, the pattern, of the organism, which is presented in this paper has developed from an extensive

¹ This paper, essentially in its present form, was written and accepted as a contribution to the proposed Williston Memorial Volume. Publication of that volume having proved impossible, the paper is dedicated to the memory of Professor Samuel Wendell Williston, both as an expression of personal regard and as a record of the purpose for which it was originally intended. The paper represents in considerable part the combined subject-matter of several addresses given at the University of Washington, the University of Wisconsin, the University of Cincinnati and elsewhere.

foundation of experiment and observation, but in the interests of brevity and in order to clear the ground, it is desirable to consider the problem in certain of its more general aspects before taking up the data on which the conception is based.

PROTOPLASMS AND ORGANISMS

The problem of pattern always involves a problem of material or substratum. The order and unity which we call pattern always appears in some sort of material of substratum. The house represents a pattern superimposed upon the material, brick, stone, iron, wood, etc., of which the house is built. The organism likewise represents a pattern of some sort, arising in some way, in the material or substratum which we call protoplasm. Each of the materials of which the house is built also involves a problem of material and pattern different from that of the house as a whole. The brick, the stone, the iron, the wood, each represents a certain sort of unity and order in a certain sort of substratum, and the analysis may be continued down to the molecule, and if we define the term "material" very broadly, even to the atom. In the case of protoplasm also a similar analysis is possible. In other words, integrations or individuations of different orders of magnitude exist. The molecule represents an integration of a higher order of magnitude than the atom, the brick, stone, iron, wood, on the one hand, the colloid particle and all the other constituents of the complex system, protoplasm, on the other, involve one or more integrations of a higher order of magnitude than the molecule. The house and the organism again represent still further integrations, and houses may be integrated into town patterns, organisms into social groups of various orders of magnitude.

From this viewpoint the organism represents an integration, a pattern of a certain order or certain orders of magnitude, occurring in protoplasm as a substratum. Moreover, since it is concerned with masses of proto-

plasm, with cells or with cell masses, it is evidently of a higher order of magnitude, *i.e.*, on a larger scale, than the pattern of protoplasm. Similarly, the pattern of a multicellular organism is of a higher order of magnitude than that of the cell. The organism is then first of all an integration of protoplasm into a unity of some sort and, in its more complex forms, an integration of simpler organisms, the cells, or of tissues and organs, each consisting of numbers of cells. In view of this distinction between protoplasm and organism the word "organismic" becomes as necessary as the word "protoplasmic." All that pertains to the higher order of magnitude of pattern and integration in the organism, as compared with the protoplasm of which it is composed, is organismic, as distinguished from protoplasmic. I have used the word in this sense in earlier publications, but it was used much earlier by Rhumbler, and Ritter has recently used the word "organismal" in a somewhat similar sense.

CERTAIN CHARACTERISTICS OF ORGANISMIC PATTERN

In the preceding section the organism has been designated as an integration. If this integration is physiological, *i.e.*, physico-chemical in character, rather than a manifestation of a metaphysical principle, as the vitalists assume, it must involve relations of dominance and subordination, of control and being controlled. As a matter of fact, relations of this sort are apparently among the most general physiological characteristics of organismic pattern. Such control is exerted, on the one hand, by the transmission of energy-changes, on the other, by the mass transportation of chemical substances from one part to another. The transmissive type of dominance attains its highest development in transmission of excitation in the nervous system, together with its receptors and effectors, and the transportative type of dominance, in the complex "chemical correlation" of the higher animals, particularly in the products of the endocrine glands. In addition to these physiological features of organismic pat-

tern, a visible spatial pattern exists or arises during development. This consists in a more or less definite order of localization and differentiation, both as regards spatial arrangement and sequence in time, of different parts, the tissues and organs. We know that the existence and development of these parts is physiologically determined, and there is every reason to believe that the morphological pattern of the organism must be in some way dependent upon its physiological pattern. In short, such pattern must represent the more stable and permanent effects or results of physiological processes.

Moreover, the capacity of the organism for regaining or approaching its original condition after disturbance of that condition appears in the morphological, as well as in the physiological features of pattern. This capacity for regulation, whether it be regulation of behavior or development of a new complete individual from an isolated part, indicates clearly enough that the organism is not simply a preestablished harmony in a mosaic of independent parts, but a real unity in which dominance and subordination are real and effective to a high degree. Even normal development, with its highly definite and constant sequence and spatial order of events, shows every indication of being, at least primarily, a controlled and ordered process. Specialization of parts may sooner or later limit their capacity for response to altered conditions by change in behavior, but such limitation does not constitute evidence against the existence of a relation of dominance and subordination in development. The facts indicate that the limitation of regulatory capacity is itself a result of this relation. When, through its relations with other parts, a part has been determined in a certain direction of specialization, it is often able to progress along the predetermined path, even after isolation from the factors which originally determined it.

Again, the relation which we commonly call coordination is in reality a complex of relations of dominance and subordination. In short, all the evidence indicates that a

fundamental characteristic of organismic pattern is a system of physiological relations of control and being controlled. These relations show all degrees of fixity. Some change from moment to moment, *e.g.*, the relations between various reflex arcs in the higher animals and man, while at the other extreme we find relations such as those associated with physiological polarity and symmetry, which either persist throughout the life of the individual as actual relations of dominance and subordination or determine directly or indirectly structural or functional conditions which are persistent. The question of the nature and origin of these physiological relations is at least an important part of the problem of organismic pattern.

THEORIES OF ORGANISMIC PATTERN

Some biologists have maintained that a problem of organismic pattern does not exist, that the organism is nothing but a mosaic of cells or protoplasmic parts. According to this view, the only problem of pattern is that of the cell or of protoplasm. This is very much as if we should say that the pattern of the house is really given in the patterns of the single bricks or stones and that, given the proper number of bricks or stones of the proper patterns, they must of necessity arrange themselves to constitute a house. We know that bricks and stones do not behave in this way, but certain biologists hold that the living cells have the inherent ability to make a multicellular organism.

So long as our knowledge of living things was based on superficial observation and introspection there was little doubt that the organism represented the working of an agency fundamentally different from anything in the inorganic world. This "vitalistic" conception has undergone modification with the advance of experimental method in biology. In its modern form it holds that the integration, the harmony, the capacity for regulation or adjustment, can be accounted for only in terms of a meta-

physical entity or principle which works in a purposive manner, that is, as if intelligent. The "neovitalistic" conceptions have the merit of recognizing that the problem of organismic pattern exists and is of fundamental importance, but all the solutions of the problem which they have offered thus far involve assumptions or generalizations which are not only premature, but which involve the more or less complete negation of scientific method.

The so-called preformistic conceptions of the organism, ranging from Bonnet's theory of "emboitement" to the Weismannian germ plasm with its determinants, ides and idants and the still more recent modifications of the conception, either assume the existence of organismic pattern in the hereditary constitution of the germ plasm, or ignore the problem. Weismann's integration of determinants into patterns of various orders of magnitude and the apparent belief of certain more recent preformists that the pattern of the organism is given in the intranuclear or chromosomal pattern all involve the assumption of the existence of organismic pattern, but throw no light on the problem of its nature and origin. The more recent preformistic conceptions are chiefly concerned with the supposed elements of the pattern rather than with the integration of these elements into a harmonious whole. It is evident, however, that if we conceive the elements of organismic pattern as discrete and persistent hereditary entities, whether we call them determinants, factors, or something else, we must also conceive some sort of controlling or ordering factor to account for the harmony and constancy of result in their developmental and regulatory behavior. But the preformistic theories have failed to provide any adequate physico-chemical basis for such an integrating factor. From this viewpoint they are to a greater or less degree vitalistic in implication, that is to say, they not only fail to give us an adequate physico-chemical basis for integration, but it appears that nothing short of a metaphysical principle,

working intelligently with all physico-chemical agencies under its control, can be conceived as adequate for the ordering and integrating into a harmonious whole the inconceivable multitude of postulated hereditary entities.

Various attempts have been made to formulate more or less strictly preformistic conceptions of the organism in terms of molecular, or intermolecular relations. Such, for example, are the suggestions that factors similar or analogous to those concerned in determining the integration and form of the crystal are the primary factors in organismic pattern and various other suggestions based on stereochemical conceptions. We even find the view advanced that the germ plasm is to be conceived as a single complex molecule. In view of the physico-chemical complexity of protoplasm, the absence of any optical or other indications of a static molecular or stereochemical arrangement in protoplasm in general, and the rôle of chemical reaction in growth, development and function, these hypotheses have not generally been found satisfactory as a basis for interpretation. Nevertheless, if a physico-chemical formulation of preformistic theories is possible, it must apparently be made in some such terms as these. If, for example, the chromosome is a physico-chemical entity and consists of a definite series of hereditary factors or genes, we can conceive it only in terms of stereochemical, crystalline or other physical order. But such a conception does not carry us far toward the interpretation of pattern in the cell or the multicellular organism: for this some sort of order on a larger scale is necessary.

The epigenetic viewpoint contrasts sharply with the preformistic in maintaining that relation between protoplasm and environment is a fundamental factor in determining organismic pattern and integration. Thus far, however, the epigenetic school has not advanced any very definite conceptions as to the nature of organismic pattern or the manner in which it arises out of the relations between protoplasm and environment. Moreover, the

high degree of constancy of course and results of development, as regards both form and function, has presented difficulties to epigenetic interpretation.

The problem of organismic pattern remains then a real problem and one which is forcing itself more and more on the attention of biologists. The following sections of this paper are devoted to a consideration of this problem in its physiological aspects, as viewed in the light of recent investigation. This consideration leads to an essentially epigenetic conception.

THE PROBLEM FROM A PHYSIOLOGICAL VIEWPOINT

Attention has already been called to the fact that the organism represents a pattern on a larger scale than that of protoplasm. It is an integration among regions or masses of protoplasm, or in multicellular organisms, among masses of cells. The cell itself is primarily an organism and the problem of its pattern is a part of the general problem. An organism consists, not simply of protoplasm or cells, but of protoplasms, cells or cell masses which behave in different ways and become different structurally, but constitute in each particular case a unity or harmonious whole, and it is this unity which constitutes the essential characteristic of the organism. If the organism is a physico-chemical entity, the integration of the protoplasm, cells or cell masses must be accomplished in some way by physico-chemical factors, but by such factors working on a larger scale than those concerned in protoplasmic constitution. In the organism physiological relations involve large areas of protoplasm or large numbers of cells and, either by means of transmission of energy changes or of mass transportation of substances, regions and parts separated by considerable distances are brought into relation. According to both vitalistic and preformistic conceptions, the organism arises independently of environment and enters into relation with it only after a certain stage is attained. As a matter of fact, however, the organism has no significance

apart from environment. Its pattern is the basis of its behavior in the broadest sense in relation to environment, and it stands in relation to environment at every point and at all times. The factors in this relation are accessible to scientific investigation, and we must first of all determine their significance for the origin and nature of organismic pattern. Only when it is finally proven that organismic pattern does not arise in relation to environment are we scientifically justified in falling back on speculative conceptions. For the present, then, the problem is a physiological problem.

In the past the distinction has not always been clearly drawn between the integrating factors in the organism and that which is integrated. Each kind of organism is made up of protoplasm with a specific constitution which we may regard as, at least in large measure, hereditary, and therefore as given for any particular individual. It is this specific constitution of the protoplasm which determines that the organism shall be a certain species, whether alga, sea urchin, dragon fly, fish, etc. On the other hand, the organismic pattern in the strict sense is not concerned in determining the particular species of organism, but merely in integrating and ordering the process of realization of the hereditary potentialities of the protoplasm so that the unity which we call an organism results. In other words, organismic pattern does not belong primarily among the hereditary potentialities of any particular protoplasm, but is a physiological factor concerned in the orderly realization of those potentialities or certain of them, in the development and function of all protoplasms.

If organismic patterns were inherent in, or associated with the specific hereditary constitution of the various protoplasms, we might expect to find a specific organismic pattern for each specific protoplasm. As regards various minor details we do of course find such specific differences in pattern, but as regards the more general features it is far from being the case. The pattern of the

cell or protoplast, for example, is fundamentally similar in animals and plants. In multicellular organisms also we find very similar patterns composed of very different protoplasms. In their more general features the patterns of hydroid colonies and of certain multiaxial plants resemble each other very closely, not only as regards form of the whole and arrangement of constituent members, but as regards the physiological factors which determine the relations of growth and form in the whole. Differences in physiological condition along the axis are very similar in both, and a physiological relation of dominance and subordination integrates the whole in essentially the same way in both. Again, the bilateral liverworts, *e.g.*, *Marchantia*, and the bilateral flatworms, such as *Planaria*, are very similar as regards the general axiate-symmetrical pattern, and in the one case the growing tip, in the other the head region, is dominant over a certain length of body.

In fact, there is apparently no relation between the more general and fundamental features of organismic pattern and the differences in specific hereditary constitution of the different protoplasms. There are in all organisms only three chief types of spatial order or pattern, as indicated by the general arrangement of organs and parts. The pattern may be radially symmetrical, referable to a point, polar, referable to a line, or bilateral, referable to a plane. Localization and differentiation of parts in all organisms, so far as we know, occurs according to one of these three orders, or some combination or modification of them. The cell is perhaps primarily a completely radiate or spherically symmetrical organism, a surface-interior pattern. Both plants and animals show various combinations of radial, polar and bilateral pattern, and asymmetries or spiral patterns appear in some organisms. Physiologically the relation of dominance and subordination is apparently the fundamental relation in organismic integration in both plants and animals. In short, the three sorts of spatial pattern

and the relation of dominance and subordination appear to be the primary features of organismic pattern, and the question is whether we can discover any physiological basis for them.

Between the parts of an organism physiological correlation exists. Leaving out of account the purely mechanical relations of pressure and tension, which are obviously not of fundamental importance, two chief groups of correlative factors are distinguishable. These are, chemical or transportative, involving the mass transportation of substances over appreciable distances, and excitation-transmission, involving first the excitation of some region and second the transmission of the excitation through a greater or less distance.

If organismic pattern is not inherent in protoplasm, it is evident that chemical or transportative correlation can not be the primary physiological factor in such pattern, because definite and orderly transportative correlation on an organismic scale is possible only when organismic pattern is already present and differentiation of different regions or cells has occurred. After such differentiation has occurred, transportative correlation becomes possible and increases in complexity and importance with the progress of differentiation, but it can not originate the pattern on which its existence depends.

Turning to transmissive correlation, several points may be noted: First, that all living protoplasm is excitable and to some degree capable of transmission; second, that excitation is not autonomous, but results in the final analysis from action of an external factor; third, that excitation and transmission may occur in a cell or cell mass without any previous specialization or differentiation of either the region of excitation or the path of transmission; fourth, that in the absence of definite conducting paths excitation undergoes a decrement in degree, intensity or energy in the course of transmission and is therefore limited in range; fifth, that the region of original excitation becomes for the time being physiologically domi-

nant over other regions within the range of transmission, because it is the initiating factor in the correlation.

In its primitive form, then, this excitation-transmission relation represents a gradient in physiological state, or degree of excitation, arising in protoplasm in response to the local action of an external factor. As long as it persists, it constitutes a pattern, a physiological integration different from any inherent feature of protoplasmic pattern. In fact, the excitation-transmission relation appears to be the most general and most primitive relation of *organismic magnitude* possible in protoplasm. If organismic pattern originates in the relation between protoplasm and an external world, it appears from what has been said that the excitation-transmission relation must be the most general and most primitive factor in such pattern.

THE SPECIFICITY OF FOOD-PLANTS IN THE EVOLUTION OF PHYTOPHAGOUS INSECTS¹

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THE great variety of foodstuffs acceptable to the insect palate is well known and accounts in part for the great abundance and variety of this group of animals. Coupled with their catholic tastes as a group is the anomalous condition that the vegetarian members of the series not only show far less latitude in selecting their food than do other herbivorous animals, but are as definitely restricted in their choice of food as are parasitic animals. These facts are so self-evident and have been so frequently commented upon that their evolutionary significance has been very generally overlooked. In fact, the present interdependence of insects and plants is so complex that the simpler relations of individual species to particular food plants seem by comparison to offer little to interest the present student of biology. If we inquire more closely into the matter, however, it is seen that they offer not only an interesting field for speculation, but opportunities for the application of experimental methods.

Quite naturally almost all our knowledge of the specificity of food-plants and their selection by phytophagous insects is based on direct observations of the behavior of a great many species in nature. Deductions drawn therefrom emphasize particularly the great nicety of adaptation exhibited by the insects, their fixed and almost immutable behavior, the delicacy of their senses in recognizing certain plants, and, finally, the coordination between the egg-laying instincts of the mother and the food-appetites of her larval offspring, however greatly their food may differ from her own.

¹ Contribution from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 233.

I have already attempted to point out in *THE AMERICAN NATURALIST* (Brues '20) and elsewhere ('23) what vast differences exist between polyphagous, oligophagous and monophagous insects so far as the extent of their diet is concerned and some of the conclusions that may be drawn therefrom. On this basis, such groups appear to be more or less fundamentally distinct one from another, although attention has been drawn also to the fact that among polyphagous and oligophagous species there are certain preferred food-plants. This at once suggests a greater plasticity in the organization of these insects which we may regard as due either to more variable instincts, less restricted powers of digestion and assimilation, or to the occurrence within species of races possessed of predilections for only certain of the food-plants that make up the diet of the species as a whole. Such a combination of physiological races is essentially analogous to the concept of impure species as composites of pure lines, except that it involves behavioristic and not morphological characters. Although there is much evidence to support the validity of such a comparison, the matter has not been subjected to the careful experimental scrutiny bestowed upon the morphological composition of species. Nevertheless, as we shall see in a moment, it appears very probable that in at least some polyphagous insects there are phytophagic races whose food-habits are clearly different and that each type is maintained through heredity under natural conditions. It is thus probable that crossing between such races does not normally occur, at least to any appreciable extent, although in polyphagous species having a wide range of food-plants there appears so far to be no evidence to indicate selective mating between individuals either from the same or from different food-plants. The small amount of evidence actually relating to known hybrids, which will be presented later, is very fragmentary and of contradictory nature.

The fact that some species of insects include groups of individuals having different food-preferences seems first

to have been recognized by the pioneer American entomologist, Benjamin Walsh, who wrote quite extensively ('64 and '65) on the subject and drew conclusions based not only on close observation of insects in the field, but also on some experiments which he performed with several species in confinement. Walsh was satisfied that there existed intraspecific groups of individuals with different food preferences, showing sometimes no structural differences, sometimes slight structural differences in the larva, and sometimes also in the imago. Such groups he believed were incipient species, having acquired a hereditary preference for certain food-plants and destined to diverge more widely in the course of time. Written so soon after the appearance of Darwin's "Evolution of Species," Walsh's account is in part combative of the doctrine of special creation, and his cases are cited with reference to evolution as influenced by isolation arising from the attachment to particular food-plants. He does not, therefore, speculate to any extent on the shifts to new food-plants, nor on the way in which such changes may have become fixed. Some of Walsh's work is cited more specifically on a later page.

After the lapse of a number of years, Cockerell ('92) presented a short note in which he attempted to explain the segregation of phytophagic types among polyphagous insects and also the shifts to new food-plants, on the basis of the principles of natural selection. A part of his thesis rests upon the occurrence of intraspecific variations that tend to feed and develop more satisfactorily on certain particular ones of the food-plants normally eaten by the species as a whole. His ideas are closely similar to those of Walsh.

During the present century a number of entomologists have given their attention to the facts just referred to, and the behavior of certain insects in relation to their food-plants has been further studied, although Walsh's papers seem to have been overlooked.²

The small Trypetid fly, *Rhagoletis pomonella*, fur-

² A discussion of Walsh's work by Craighead ('23) has since appeared.

nishes an unusually clear case of a single species which is represented by two distinct forms or races that are restricted to different food-plants, although they show absolutely no morphological differences except for a quite constant difference in size. This insect appears to be undoubtedly native to North America, and it seems very probable that it originally bred in the fruits of certain species of *Crataegus*. On account of its great economic importance, it has been carefully studied by several entomologists, Comstock ('82), Harvey ('90), Illingworth ('12) and O'Kane ('14).

The apple-maggot has practically deserted its original food-plant and is now a very abundant and destructive pest of apples in New England, New York and Pennsylvania, extending also into the Middle West in much decreased numbers. The larvae mine within the fruits, especially those of the summer and early fall varieties. Several times during the past twenty-five years this insect has been reported as breeding in blueberries, and Woods ('15) has given a very entertaining and instructive account of a smaller race which is a common blueberry insect in some parts of the state of Maine. Woods finds noticeable differences in size in both the adults and larvae from the two plants; thus, the male flies from apple average 4.60 mm, those from blueberry 3.60 mm, while the females average, respectively, 5.80 and 4.20 mm in length. Similarly, the larvae are distinguishable on the basis of size, and although there is much variation in each series, in no case is there any overlapping between the two races. Furthermore, he finds that the smaller blueberry flies are much more active and more wary in their behavior than those from apple. Attempts to transfer the apple race to blueberry or *vice versa* were unsuccessful, even in the case of half-grown blueberry-maggots transferred directly into apples, and Woods concludes that he "is inclined very strongly to believe that biologically at least there are two distinct strains or races of *Rhagoletis pomonella* Walsh, the one breeding in apple and related fruits, and the other in smaller

fruits such as the blueberry and huckleberry. There does not seem . . . to be any other conclusion which will explain the data given above. Certainly, in so far as *Rhagoletis* occurs in Maine, the form on the apple and the form on the blueberry are entirely independent. The oldest inhabitant of the barrens can not remember a time when there were not maggots in the blueberries, while the introduction and spread of the apple maggot in the state is a matter of record. . . . In Maine the blueberry maggot apparently did not migrate to the apple nor *vice versa* and the two races have lived on independently side by side."

The leaf-beetle, *Calligrapha scalaris*, feeds on a number of plants, and Walsh ('64) noticed that there are two types distinguishable by their size. The larger one, measuring 7-8 mm in length, occurs on elm and basswood, and the smaller, 5.5 to 6 mm long, he found on dogwood and wild plum.³

The quite sudden adoption of a new food-plant by the common butterfly, *Papilio zolicaon*, in California appears to be well authenticated (Coolidge '10). Like *P. asterias* of the eastern United States, *P. zolicaon* feeds upon various Umbelliferae, especially celery, parsley and Carum, but has been found abundantly in at least two localities in California on citrus fruit trees. Although this is a new habit for this species, the change may be in the nature of a reversion, since another group of *Papilios* of wide distribution feed on citrus.

The well-known codling moth, *Cydia pomonella*, is an abundant species of holarctic distribution which develops in the larval condition in the fruit of the apple. There is a race or form of this species, however, which feeds in walnuts and has become a great pest in certain parts of California where walnuts are extensively grown. In Europe a walnut form is also known, and this apparently is the one now found in California, as it does not appear

³ On account of the difficulty of distinguishing the species of this genus, Walsh's observations are not conclusive (*v.* Knab '09). *C. philadelphica* feeds on Cornus.

that the insect has migrated directly from apple to walnut, although there appear to be no morphological differences between the two races (Foster '10; Smith '18).

The common alder flea-beetle of the eastern United States, *Haltica bimarginata* Say, feeds almost exclusively on the leaves of the alder, *Alnus incana*, both as a larva and adult, although it sometimes occurs on willow, poplar and cottonwood. Woods ('17) found it in Maine regularly on alder, and at one single locality on the balsam poplar (*Populus balsamifera*). Larvae obtained from eggs deposited on alder fed readily upon alder, willow and aspen (*Populus tremuloides*) but absolutely refused to eat leaves of the balsam poplar, although individuals obtained from balsam poplar readily accepted this plant as well as alder, aspen and willow. It appears evident in the case of this beetle that alder is the preferred food-plant for the species, but that a strain exists, at least in one locality, which will accept the balsam poplar, although it has not developed a distaste for alder.

The abundant and well-known caterpillar of *Lasio-campa quercus*, which formerly fed almost only upon oak, is now commonly found on a variety of widely different trees, and similarly *Abraxas grossulariata*, formerly restricted to Ribes, now feeds regularly on the foliage of oak, Euonymus and other woody plants.

That the acquisition of apparently new food-plants may really be a reversion or return to one formerly selected during the phylogenetic history of the insect appears probable in some cases. Thus, in France the nun moth lives almost exclusively on oak, but feeds on pine in Germany. Pictet ('05) regards the latter habit as a reversion to an ancestral food-plant, and if such be true the acquired oak habit may be regarded as probably kept up by a racial memory, liable to be overcome at any time by a more deep-seated phylogenetic tendency. We will see later that such a supposition finds some support in experimental work on racial memory extending over only two or three generations.

Examples like the *Rhagoletis*, *Papilio*, *Cydia* and *Haltica* just cited, have in some cases been explained not as sudden changes or mutations in habit, but as having an evolutionary or historical significance. Thus, Vassiliev ('13) during a study of beet-insects in Russia found that a weevil, *Bothynoderus punctiventris*, regularly attacks two species of *Chenopodium* and one of *Atriplex* in addition to the beet (*Beta vulgaris*), all members of the Chenopodiaceae, but that it feeds also on *Polygonum aviculare*. Since the Chenopodiaceae and Polygonaceae are related, the author assumes that the weevil originated at a time when the relationship between these two groups of plants was closer, with more intermediate forms. This assumption necessitates a very rapid evolution of plants and corresponding inhibition of change in insects and does not receive any paleontological support.

Parallel evolution of insects and their food-plants where groups are concerned rather than isolated species is, however, much more plausible and the probability of this having taken place in many instances appears greater as we examine in detail the food habits of certain insects. In the case of the violet-eating Argynmid butterflies and similar groups, it appears very evident that evolution has proceeded concurrently among the plant and insect species. The Pierid butterflies are essentially feeders on Cruciferae, but a few genera affect Leguminosae. The family is widely distributed and has produced many forms restricted to crucifers, some living on Leguminosae, and a few, *Catopsilia* and *Callidryas*, restricted to the single genus *Cassia* in widely separated parts of the world. We can not be far amiss if we assume that the acquisition of a leguminous food-plant was a mutation or at least a sudden shift and that diversification of the insects has proceeded not necessarily with any reference to that of the plants, but that the two have gone on side by side, most of the insects gradually acquiring additional, although not necessarily very closely related, leguminous food-plants.

A most unexpected change in food habits was observed by Pictet ('11) in the course of experiments with the caterpillars of *Lasiocampa quercus*. Larvae of this species derived from oak, which, together with other deciduous trees, forms their normal diet, were placed on pine. Of these many died, as they could not open their jaws widely enough to remove the tissue from the pine needles. Some, however, survived by feeding at the ends of the needles where they can work into the parenchyma. The second generation was then found to be adapted to pine, on which the caterpillars fed without difficulty and furthermore they were unable to return to oak leaves, which they attempted to enter from the tip as their parents had done on the pine needles, although the larvae are normally edge feeders. Pictet's experiments certainly suggest that memory has played a part in the behavior of the second generation. Nevertheless, it is clear that there has been a selection of a few individuals in the first generation and that their offspring may be expected to inherit at least the power of adaptation to pine shown by their parents in adopting a new method of feeding. On the other hand, we should expect the offspring to be equally quick in readapting themselves to oak, which they do not appear to be. One other supposition may be made also, that there was a drastic selection in the parents which actually changed the form of the mouthparts sufficiently to make oak-feeding difficult. This latter does not seem very likely, but makes it necessary to regard Pictet's experiments as not entirely conclusive.

The food-preferences of a small leaf-mining fly, *Pegomya hyoscyami*, have been studied in England by Cameron ('14 and '16), who finds that within this species there are groups which react differently toward the several food-plants upon which the larvae may develop. As the specific name indicates, the henbane (*Hyoscyamus*) serves as one food-plant, but another solanaceous plant, *Atropa belladonna*, is attacked as well as certain Chenopodiaceae, such as *Chenopodium album* and the cultivated beet and mangold.

It appears that ordinarily when henbane and belladonna are grown in proximity, the former is extensively damaged by the flies which avoid the belladonna, but that when henbane is absent the flies are attracted to belladonna, on which their larvae develop. Also, flies reared on belladonna will oviposit and develop on mangolds, if deprived of the parental food-plant; but, on the other hand, oviposition on beets by individuals reared on mangolds did not occur. These experiments could not be carried sufficiently far to demonstrate the behavior of the several broods toward different food-plants, but they show clearly the existence of a preference for the parental food and a varying ability or willingness to shift to others. In connection with this species it is worthy of note that the several forms of *Pegomyia*, some of which are well-known noxious insects, have extremely diverse habits, indicating a great plasticity of behavior and high degree of adaptability in the genus.

The change of an insect from one food-plant to another with a coincident change in structure has been reported by Marchal ('08) in the case of the soft scale, *Lecanium corni* (= *persicae*). The evidence, which appears to be well substantiated, is in brief as follows:

Marchal was led to believe from the presence of a *Lecanium* on the American tree, *Robinia pseudacacia*, naturalized in Europe that the insects had been derived from some native palaearctic species. The form on locust (*L. robiniarum*) is now scattered through various parts of France, but was unknown previous to 1881. Likewise, it was first found in America in 1892 by Cockerell in New Mexico far from the Appalachian ridge that represents the original habitat of *Robinia pseudacacia*. How extensive the insect may now be in America I do not know, but it was present near Boston in 1912.

By taking eggs from individuals of *L. corni* on a peach tree, and transferring them to locust, Marchal succeeded in establishing a brood upon locust and the adults on maturing showed "the large size, dark coloration and definitive habitus of *L. robiniarum*."

Attempts to restore these insects to peach were futile in the single experiment made by Marchal, and he concludes that the inverse change of food-plants is at least more difficult to make, although it may be possible.

Some experimental evidence relating to the memory of leaf-eating caterpillars, as indicated by their reactions toward undesirable food-plants, has been presented by Mayer and Soule ('06). They found that in the case of the common milkweed butterfly (*Anosia plexippus*), larvae that have commenced to feed upon a milkweed leaf may be induced to bite at leaves they would not normally eat if these be presented to the feeding larva at intervals of not less than a minute and a half. In such cases the larva takes the same number of bites each time after repeated trials, showing that it exhibits no distinct direct nor cumulative memory of such experiences. If, however, the unacceptable leaf be offered as frequently as each half minute, the number of bites taken decreases with each successive experience. This cumulative effect soon results in complete refusal to bite at a distasteful leaf.

Just how far such experiments bear on memory in relation to the selection of food is by no means clear. They show that the process of feeding in such caterpillars proceeds in a very mechanical way after it is once initiated and well in progress, that is to say in the case of the *Anosia* larva, after feeding for one and a half minutes, and that eating stops only after the different flavor is recognized by the insect. They show also that the larvae do not apply their previous experience in dealing with a specific problem when it is again presented after one and a half minutes, although the same caterpillars appear to do so if the occasion arise at the expiration of a half minute period. This is not in any sense the memory of a particular flavor or odor which must be the stimulus to be taken into account in any theory of associative memory applied to the selection of food-plants by adult insects that have fed in the larval stage upon particular

plants, and does not seem to shed any light on this question. That such is the case is also shown by the fact that the caterpillars in the experiments reacted in an identical way to strange leaves, paper and tinfoil, and thus regarded the world as composed of either "milkweed" or "not milkweed," just as the cabbage-worm arbitrarily divides its world into Cruciferae and other objects too numerous to mention.

A recent paper by Craighead ('21) contains a very interesting report on experiments upon longicorn beetles, undertaken to determine the possible modification of food-plant selection resulting from continued breeding in the wood of particular species of trees. For this purpose Craighead selected a series of beetles each known to have more than one food-plant in nature and examined the behavior during subsequent generations of strains of each species bred upon the several food-plants. Eleven species of beetles belonging to eight genera were used in these experiments, some confined almost exclusively to a single food-plant, some to two or three plants and some which attack a rather wide variety of trees.

Thus, *Cyllene pictus* feeds almost exclusively in hickory, although rare instances are known of its occurrence in grape, mulberry, osage-orange and hackberry. It was found that this species when obtained from hickory will readily adapt itself to grape and mulberry, and also to oak, although this does not appear to be a natural host. Furthermore, in the case of mulberry it was found that the hickory strain after breeding for one generation in mulberry selected the latter wood for oviposition in which the larvae then matured successfully. A similar transfer of this same strain to grape gave a somewhat more doubtful result. At this point, however, we must indicate some of the difficulties attendant upon these experiments. The selection may be influenced by the condition of the wood (as regards seasoning, moisture, etc.) and also by the amount of available material of each, since it appears evident that adults will prefer a secondary host to overinfesting the preferred one. Such dif-

faculties are unavoidable, but can, of course, be minimized when they are thoroughly understood. Attempts to transfer this species to locust (*Robinia pseudacacia*) and ash (*Fraxinus*) were unsuccessful;⁴ oviposition occurred, but all the resulting larvae in ash died as well as most of those in locust and as imagines resulting from the latter did not reproduce, they may have been sterile. A number of larvae from hickory, transferred to ash after they were half to three fourths matured, developed in the ash, but the resulting beetles appeared also to be sterile, as they failed to produce larvae when caged over both ash and hickory.

With the strain of this species obtained on grape, it was found that the first generation selected grape, but that the next generation attacked both kinds of wood.

Callidium antennatum, a species that occurs in seasoned pine, is known to occur in spruce also, but a strain from pine failed to reproduce in spruce when caged over both woods, although a single larva (which later died) was found in the spruce. Later in the season, Craighead transferred some half-grown larvae to spruce, in which they completed their development, and the emerging beetles in great part selected spruce the following spring and entirely so in the next generation the ensuing year.

Xylotrechus colonus is a widespread, polyphagous species that occurs in nearly all the hardwood trees of the eastern United States. A strain of this beetle obtained from oak showed a decided preference for oak, chestnut and hickory. It was barely able to maintain itself in ash and maple, but totally unable to complete its development in locust. By isolating from the original oak strain, separate series on oak, chestnut and hickory, after several years strains were obtained which exhibited a growing preference for the particular wood to which they had been restricted.

⁴ Walsh ('64) regarded the locust form (*C. robiniae*) and the hickory one (*C. caryae*) as phytophagic varieties, but they are now generally considered as distinct species in which the specific differences are most clearly marked in the male.

The data obtained from the other species agree well with those cited above, although some were not so clear-cut. Although these experiments are not so complete and convincing as might be desired, they at least strongly support the validity of several conclusions. There can be no reasonable doubt that there is a general tendency for the individual beetles to oviposit more abundantly in the species of wood in which they themselves fed as larvae than in another which is from the standpoint of the entire population of the species more desirable. Furthermore, there appears to be an increasing degree of preference after additional generations, although in one case, that of *Callidium*, the adults of the third generation suffered a relapse, and, for some reason not evident, reverted to their original diet of pine. Considering the nature of the case, however, and the fixity of food-habits in general, reversions of this sort must be expected and if they do not invariably occur they do not disprove the conclusion expressed above. A second fact brought out by these experiments is that oviposition frequently occurs on woods that are not suitable food for the larvae, which consequently suffer a high or even complete mortality at an early age. This is in accordance with observations made on certain parasitic insects, cited in a previous paper (Brues, '21), where certain unsuitable hosts may serve for oviposition, but not for the successful development of the parasitic larvae.

Another instance which may possibly be interpreted as supporting Craighead's contentions has been reported by Hegner ('10). This relates to a Chrysomelid beetle, *Calligrapha bigsbyana*, the larvae of which feed normally on leaves of *Salix longifolia*. However, when Hegner reared larvae of one generation of *Salix amygdaloides*, their offspring were found to show no preference for their natural food-plant, but attacked both species of willow without preference.

An experimentally obtained shift of food-plants, involving not only considerable mechanical difficulty, but

finally resulting in a different method of feeding, has been reported by Schroeder ('03) in the leaf-beetle *Phratora vitellinae*, the larvae of which feed on the leaves of the willow, *Salix fragilis*. The leaves of this willow are smooth beneath and are skeletonized by the larvae which feed upon the underside, leaving intact only the superior epidermis. Larvae transferred to *Salix viminalis*, which has the leaves densely downy beneath, found feeding difficult, but the insects were able to push this material aside and reach the parenchyma of the leaf, and one actually avoided the pubescence and excavated a mine in the leaf-tissue. After four generations on the downy willow the larvae had gradually adopted the leaf-mining habit, and showed a greater percentage of choice for *S. viminalis* as the proportion of adults that selected this plant for oviposition increased from 9 per cent. to 42 per cent.

The results obtained by Schroeder appear to be strictly comparable to what might easily occur in nature as the result of the continued scarcity of a preferred food-plant.

Several observers have called attention to an ontogenetic cycle of changes which frequently occurs among the most diverse insects whereby there is a shift from one food to another during larval development. Cases where there is an extreme shift in food-habits associated with profound morphological changes do not concern us here, as they have a clearly structural basis. Those where the shift is less and involves merely the migration to a different part of the food-plant have a more direct bearing on the present discussion and I shall return to them in a moment. Quite similar to these is the phenomenon of alternation of generations exhibited so clearly in many hymenopterous gall-insects of the family Cynipidae. This involves also great structural differences in the insects and so many other as yet undetermined factors that its bearing on ancestral memory and related questions remains very obscure.

In some other groups of phytophagous insects, however, we find what appears to be an incipient alternation

of generations in very simple form, associated with a definite alternation of food-plants. For example, the European geometrid moth, *Tephroclystis virgaureata*, passes through two generations during the course of the season, the first brood of caterpillars feeding in the early spring on Compositae (Senecio and Solidago), and the second brood in midsummer feeding on Prunus and Crataegus (Klos '01). Furthermore, the adults of the two generations show small but constant differences in color. Many other similar cases are known, and undoubtedly the phenomenon is quite widespread among multivoltine insects, including some in which the food-plant is the same during successive generations. Such a condition prevails among numerous butterflies which exhibit a seasonal dimorphism related to, and perhaps entirely dependent upon temperature. Other cases of polymorphism, on the other hand, are known to be due to the different genetic constitution of the several types. The polymorphic females of one *Papilio* which have been described by de Meijere ('11) exhibit a Mendelian relationship to one another and are thus not in any way related to temperature nor to food-plant, particularly as all forms feed upon Citrus. Where there is an alternation of food plants it is evident that those of one generation must be primitive and those of the other more recently acquired, or else the insects in question originally fed upon all the plants concerned. The latter does not appear probable since the plants concerned are usually present at both times and it is difficult to see how any sort of memory of food-plant can be repressed in the first succeeding generation, later to be awakened in the second. Nevertheless, the behavior of the *Tephroclystis* appears to indicate such a temporary repression, if memory is concerned at all in the process of selection. The acquisition of new food-plants has actually been noted in a number of instances among various insects, as we have already seen.

The effect of hybridization on the selection of food plants by larvae of the first filial generation has been observed by Göschen ('13) in geometrid moths of the genus

Celerio. The European *Celerio euphorbiae* is represented by two subspecies or varieties known as *euphorbiae* and *mauretanica*. Both forms normally feed on Euphorbia and will not accept willow, but the hybrid *euphorbiae* ♀ × *mauretanica* ♂, known as *wagneri* and the reciprocal cross known as *turatii* both feed readily upon willow and can be raised to maturity upon it. Likewise, the hybrid *C. kindervateri* (*euphorbiae* ♂ and *gallii* ♀) feeds well on willow in the first generation of caterpillars, although *gallii*, like *euphorbiae*, does not naturally occur on any plants except Euphorbia, Galium and Epilobium. However, Schulze ('13) mentions several cases where isolated caterpillars of *C. euphorbiae* have been found on Polygonum, Syringa, Plantago and Solanum. Another instance in which Schulze reared upon willow a caterpillar found on Euphorbia is particularly interesting as this individual later grew to resemble *C. gallii* closely and probably represented a natural hybrid between the two species.

That hybridization does not always affect the selection of food-plants as has just been described is shown by some observations of Field ('10) on hybrid butterflies of the genus *Basilarchia*. In this case, three forms are concerned, *Basilarchia astyanax*, *B. arthemis* and *B. proserpina*, of which the last is considered for good reasons to be a hybrid between the first two. Field found that caterpillars produced by a *proserpina* captured in the open refused birch, poplar and willow, the preferred food of *arthemis*, but were successfully reared on wild cherry, one of the favorite food plants of *astyanax*, although the butterflies reared from them represented all three forms, *arthemis*, *proserpina* and *astyanax*, and he suggests on a numerical basis that they are derived from a *proserpina* (hybrid) × *arthemis* (color recessive) mating, especially as *arthemis* occurs commonly where the specimen was taken and *astyanax* does not. In this case the selection of an *astyanax* food-plant is quite unexpected and must be derived from an *astyanax* grandparent, although only one quarter of the butterflies reared from the brood of caterpillars proved to be *astyanax*. Unfor-

tunately, he was unable, on account of the impossibility of mating the butterflies in captivity, to study the food preferences of later generations.

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RESPIRATION AS A FACTOR IN LOCOMOTION OF FISHES

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THE accepted explanation of the locomotion of fishes not in contact with some solid surface is in terms of, first, the sinuous movement of the body, and, second, the movements of the various fins. The relative importance of the numerous factors embodied in these of obvious necessity differs from species to species, depending upon the degree and kind of specialization of any given form. While the present writer professes disagreement with the accepted explanations on several points as to the manner in which the known factors react on the surrounding element, it is not the purpose of this paper to attempt an overthrowing of these current conceptions, its purpose being simply that of calling attention to the existence of another factor in fish locomotion which had escaped the observation of students until discovered and called to my attention by Mr. Clifford M. Paxton of Brooklyn, New York.

Mr. Paxton has invented and claimed patent rights on a strikingly novel method of propelling ships which he calls an "induced stream line system." His thoughts, as developed, were the results of the contemplation of abstract hydraulic principles, but his discoveries and the developments of these principles caused him subsequently to conclude that fishes must receive locomotor benefit from the automatic operation of certain parts in accordance with one or more of the principles which he has developed for the high speed propulsion of vessels. A brief consideration of Mr. Paxton's propulsion method is necessary to a proper understanding of some of these newly discovered factors of fish propulsion.

The movement of a vessel is chiefly impeded by three obstacles, as described below:

(1) *Bow-wave*: When a ship is propelled through the water all the submerged surfaces of the entrance section are subject to the adverse pressure of the relatively stationary water which has to be forced out of the way to make room for the advancing hull. This water is projected away from the ship and represents lost energy. As the ship moves, other water has to flow in by gravity to fill up the space the hull moves out of. At low speed this is not a serious matter, as the water is moved slowly and has ample opportunity to readjust its level by gravity flow. At higher speeds, however, this is not the case and the water is "pushed and piled up" in the form of a bow-wave about the entrance portion of the ship.

(2) *Cavitation*: Likewise, in reference to the after portion of the ship, water can not flow fast enough in response to gravity to instantly fill in the space vacated by the ship, and there result hollows or "low pressure areas" about the run or after portion of the hull. These augment the retarding effect of the high pressure created forward to the end that there results a pressure differential with a large rearward component, which is the dominant limiting factor in the speed of ships.

(3) *Skin Friction*: The frictional resistance between the ship's surface and the water through which it is forced is given this term. While it is not a limiting factor and does not increase in the same ratio to speed as the pressure differential does, it is, nevertheless, an important item of resistance.

The first two of these resistance factors are as great or greater in the case of a submarine vessel (or a fish), although the adverse pressures are not ordinarily visible as waves on the surface.

With an apparently simple but nevertheless very ingenious arrangement of "developed" jets, Mr. Paxton has greatly reduced the retarding effect of these factors and at the same time has been able to develop sufficient reactive thrust to overcome the remnants of resistance that still remain. The invention has progressed beyond the theoretical stage, so the inventor's actual model will be described in lieu of a necessarily longer exposition of the abstract principles. This model is nearly an exact reproduction of a modern destroyer, Class 186, reduced to an overall length of thirty-four and one half feet on a scale of 1 to 9. On each side of the hull, midway between the waterline and the keel, a short distance aft of the bow a rearwardly directed nozzle is located, each having an orifice three and three twentieths inches long and five

thirty-seconds of an inch wide. They are so placed as to cause water expelled therefrom to sheathe the underwater hull a short distance aft of them, completely surrounding the hull at midship. The position of the intake orifice is of slight importance, usually being placed where most convenient and presented forward. At this point certain principles concerning the behavior of jets may be mentioned. Contrary to popular belief, the water set in motion surrounding a stationary submerged jet moves slowly in at right angles to the edge of the moving stream and then on contact passes along with it at a velocity only slightly inferior to that of the jet. This induced flow causes more water to move with it in a similar manner, and so on, thus spreading out the stream rapidly. The initial jet increases in sectional area by its deceleration to which is added the constantly increasing induced flow. Paxton finds that with jets of high velocity the cumulative stream as thus built up may be more than a thousand times the cross section of the initial jet stream. The truth of the foregoing has been satisfactorily demonstrated by experiment.

Another fact to be here noted is that a stream ejected along the side of a curved form will follow the contour presented, even if the curve is convex to the axis of the stream, provided it is not too abrupt. The stream does not veer off at a tangent as might be supposed, but closely follows the bending of the curve. With these considerations in mind the manner in which Paxton overcomes the three obstacles to the speed of vessels by his method may be considered.

Through the nozzles described he pumps a small quantity of water at a high velocity and neutralizes the three impediments to progress, as will be shortly described. This system of propulsion is not to be confused with many which have appeared from time to time that were based on nozzle reaction and used a large volume of water at comparatively low velocity with the orifice located elsewhere. All such have been proven to be less efficient than

the modern screw propeller. One important difference is that Paxton makes the lengths of the pressure reducing and pressure increasing portions of the jet stream correspond, that is, "fit," within limits, the respective lengths of the bow and stern sections of the hull.

(1) *Bow-wave*: This may be lowered slightly by a certain amount of water passing into the propelling system through the intake orifice, but this is wholly inconsequential, since possibly less than one half of one per cent. of the water moved away from the high pressure region forward is taken into the ship. The discharge nozzle slits are so located as to be in advance of the maximum pressure region and by virtue of the water movement induced by the sheet discharged through these slits the bow-wave is in practice actually eliminated.

(2) *Cavitation*: This posterior depression is filled by both the water ejected from the jets and the flow induced by them, so that the wake trends somewhat rearward instead of forward.

(3) *Skin Friction*: Considered as resistance to the ship's motion, this is largely overcome in that a considerable part of the frictional surface is transferred to the surface of the jet stream that sheathes the hull, for this may be considered practically as part of the vessel while it still follows the contour of it.

The ship as a whole reacts to the rearward movement of water about it and in addition there is the propelling effect of the nozzle reaction ample for any remnant of resistance. The reason for giving the jets a long narrow section instead of a circular one is for the purpose of placing a larger surface area in contact with the adjacent water and sheathing the hull satisfactorily; as well as concentrating the movement, reducing the time required for it, and making the jet stream "fit" the hull. According to Paxton the modern screw propeller is more efficient than his new method at very low speeds, but at relatively high speeds the relationship is reversed.¹

Returning to the fishes, it should now be obvious that the water ejected through the gill clefts of typical Acanthopterygians is extremely similar in its effect to that of this new mechanical device that has actually propelled a model successfully. Experimentation has not been suf-

¹ All statements concerning his invention have been personally checked and approved as correct in a general way by Mr. Paxton, although naturally sketchy and inadequate for a full understanding of his invention as applied to ship propulsion. They are, however, sufficient for our purposes here.

ficient as yet to demonstrate just what locomotor advantage a given species may obtain from the water it ejects from its exhalant orifices, but the connection is evident and certain things have been proven which, together with certain indications, give food for some rather interesting reflections. An outline of the general trend of the writer's studies thus far is given below.

Recognition of the excellent but forgotten work of J. S. Brugmans is here made as he well recognized the possible importance of the influence of respiration on the locomotion of fishes. He, however, failed to comprehend the far-reaching significance of the reversal of pressure differentials and confined his thoughts to the simple reactive effect of the jets of exhaled water. His conclusion was that this effect was more important than any of the body and fin movements. No paper so far located since his time has even mentioned Brugmans' work or suggested independently the possibility of respiration having some effect on movement.²

An examination of over 300 diverse species of free swimming fishes taken at random (including both Teleosts and Elasmobranchs), most of which move at a considerable rate of speed, shows that over 90 per cent. possess gill clefts at a place which Paxton pronounces to be the theoretically correct position for the most efficient use of this method, as far as he could tell from available material, considering the varying forms. The remainder consist of few slight variations, none of which are large. Furthermore, even in sluggish forms the gill slits hold these positions fairly closely. It is only in such fishes that have progressed far from the typical ichthyized form that any wide variation is seen. Prominent among the latter are such highly specialized fishes as *Hippo-*

² Appreciation is here expressed to Miss F. La Monte, of the Department of Ichthyology, American Museum of Natural History, for translating the paper. "Aanmerkingen over de middelen door welke de visschen zich bewegen in het algemeen en over het vermogen der Uitademing tot dat einde in het bijzonder." Brugmans, J. S., Verh. l. Kl. Nederl. Inst. 1812, pp. 185-217.

campus, *Histrio* and *Lophius*. In the fast moving forms long, narrow, gill clefts are the rule, as typified by *Seriola*, *Scomber* and *Pomatomus*, while in the more sluggish forms small and often nearly circular exhalent pores are common as in *Spheroides*, *Balistes* and *Lactophrys*. Practically all intergrades are found between the two extremes which are beautifully correlated with other locomotor structures and known habits. Even in fossil fishes this correlation of the location of the gill clefts and the general body form holds good to such a degree that mere accidental association is out of the question. It is naturally difficult to obtain a measure of the force of exhalent water from living fishes moving at their higher rates of speed and at the lower rates perforce used in confinement, the body and fin movements which may be roughly analogized to a screw propeller have a great advantage. However, on the sluggish forms a definite demonstration of this force of the exhaled water is a simple matter, the Tetraodonts demonstrating it most clearly, although it must be admitted that here, on account of the rather wide divergence of the exhalent apertures, "nozzle reaction" plays a relatively larger part. Of these, *Chilomycterus schoepfii* (Walbaum) shows its ability in this direction better than any others so far examined. It is simply necessary to hold an individual of this species with its mouth immersed to observe this. Having little flexibility of body, it is unable to squirm about and necessarily confines its attempts to escape to violently lashing the caudal, anal and ventral from side to side and waving the pectorals about in addition to squirting powerful jets of water through the gill orifices. In a fish six inches long these jets may attain a height of considerably over two feet above the surface of the water. That these jets are of great use in locomotion there can be no doubt. In fact, specimens of this species have been seen to impel themselves forward through the water by this means alone at not much less than top speed. Besides being able to move themselves forward they have control of the ejections to such an

extent as to be able to effect turns by simply closing the proper aperture. If a sudden stop is desired they may close both valves and return the water through the mouth, but this is secondary to the brake-like effects of the pectorals thrown out at right angles to the axis of motion. A *Diodon hystrix* Linnaeus, with a length of about twenty inches, showed these same effects in a proportional degree, but on account of its inclination towards inflation was awkward to work with, although the results were more spectacular. It might be here called to the attention that submerged jets of pure water are perfectly invisible, and it is only when a suspended particle is acted upon by the jet that its force may be noted. Fishes that were held perfectly rigid so that there was no fin or body movement whatever appeared to be unable to eject jets with any force, simply respiring lightly, but as soon as the slightest tremor was permitted in the body the water was expelled violently. This suggests the possibility of a sympathetic nervous connection between the trunk and tail movements and respiration. Fishes of high speed, such as *Caranx* and *Seriola*, which could only be held with difficulty in a manner similar to that described for *Chilomycterus*, failed to respond appropriately, either flapping violently, or not respiring or if so only feebly, in such a manner that nothing could be deduced therefrom. The powerful *adductor operculi*, together with the branchiostegal rays and other compressible parts of the head, must make it possible for these species to eject water with a considerable velocity if so desired, and it may be mentioned that Paxton maintains that there is ample water ejected from fish held under such conditions to effect the purpose, the difficulty apparently being one of the velocity of ejection, which the musculature of the opercular and mandibular region could easily effect, were it not for some nervous inhibition incident to restraining the fish.

Probably the most satisfactory evidence in regard to these forms thus far obtained has resulted from the ob-

servation of specimens remaining quiet and freely suspended in the water. Various explanations have been given from time to time concerning the functions of the pectorals which usually are waved about rhythmically during periods of rest. The most generally accepted one being that it is done to maintain equilibrium, in that it is not done by fishes resting on the bottom. This theory has had the support of experimental evidence based on the removal of fins, it being alleged that fishes list to the side from which the fins have been removed and that with the removal of all paired fins the fish turns ventral surface up as a dead one.³

Repetition of these experiments failed to substantiate these statements as the results were directly in contradiction. Over a dozen diverse species (including these used by previous investigators) were experimented upon, varying between such extremes in form as the log-shaped *Esox reticulatus* (Le Sueur) and the deep, thin-bodied *Vomer setapinnis* (Mitchill), but the full results of the experiments will not be related here. Suffice it to say that whilst fishes with various combinations of paired fins removed were embarrassed in maneuvering in different ways, depending on what ones were missing, in no case was a disturbance of equilibrium obtained. As long as the individual remained at rest and attempted no turning or other maneuvering it retained the normal horizontal position.

A superficial glance at nearly any typical fish poised quietly in the water will convince any one that the pectorals are engaged in backing water. That is, the effective thrust is forward, which would tend to move the specimen in a backward direction. In connection with this newly described factor in locomotion it is conceived that the function of this movement of the pectorals is to neutralize the force of the exhaled water. It is not to be understood, however, that any considerable force is felt

³ See R. C. Osburn, "The functions of the fins of fishes," *Science*, 1906, n.s. 23, pp. 585 to 587. Also the Cambridge "Natural History," Vol. VII, p. 353.

from the exhalations while at rest, as naturally the respiration is slower and furthermore the gill clefts are observedly cracked wider at such times, thus reducing the velocity of the emerging stream as well as increasing the cross-sectional area of it and consequently reducing both its velocity and surface area per unit of volume. The pectorals being usually placed directly behind the gills are enabled to intercept the stream and check the original direction of the thrust. The truth of these assertions is by no means simple to demonstrate owing to the large number of locomotor organs that generalized fishes employ either single or in numerous combinations, usually seven fins besides the body movements. In fact, it is rather seldom that fishes are seen free in the water with no apparent motion other than the pectorals. At such times they are seen to back water rhythmically and usually in perfect synchronism with the respiratory movements. That is, as the pectorals come forward the operculum lowers and forces the vitiated water out, the inhalation accompanying the return stroke. Usually, however, there are some other movements such as undulation of the dorsal or caudal as well as various others which sadly complicate matters so that it is a matter of patient waiting for a proper opportunity to see these two factors working together alone in direct opposition to each other. The removal of a single pectoral of a specimen of *Lepomis pallidus* (Mitchill) demonstrated this still further. On composure after release it backed water as usual with the remaining pectoral fin, but as the force applied was only one half of that previously used and on one side only, the fish moved forward, slowly curving toward the side possessing the fin. This motion appeared to disturb the specimen, causing it to speed up the number of oscillations. As now the force of the fin overcame that of the jets the fish moved slowly backward and curved slightly to the opposite side. In a short time the fish learned to compensate for the missing member by waving the posterior tip of the soft dorsal which it bent

towards the side of the missing pectoral, and from then on had no difficulty in maintaining any position desired. Most of the Centrarchidae use either or both median fins in this manner occasionally, making the learning of this accomplishment no new feat. On this account also it is usually done by specimens practically immediately on coming to rest. The particular individual described above probably represents a slight subnormality in nervous adjustment.

Even ignoring these experiments it is still indisputable that any jet expelled from a floating body must tend to move it in an opposite direction, if simply by plain reactive force as Brugmans clearly indicated; so, when such other factors enter as the reduction in resistance, as mentioned previously, it is reasonable to suppose that the actual thrust obtained would be of some practical value to ichthyized forms. It is almost needless to add that while fishes below the surface do not throw up a bow-wave or dig out a cavitation behind, the differential pressure areas have identical effects, but are not ordinarily visible as but a single medium is involved. The reduction of skin friction is probably negligible in fishes on account of their effective mucous coat, but the other two and more important obstacles to speed must be overcome by muscular action.

Looking at the question from a phylogenetic standpoint there seems to be no very good reason why the port of exhalation in such diverse animals as Elasmobranchs and Teleosts should have such a community of placement, unless there is a positive advantage to be attained by so placing them or a definite disadvantage in having them placed anywhere else. To assume the latter is simply to euphemize the former, for as we have shown in the previous paragraph any jet of such nature must have its reactance, however slight. If there was not some sound advantage in ejecting water forcibly it would seem a useless expenditure of energy on the part of many fishes while swimming to pump water in and out when

by simply opening the mouth a greater amount would flow over the gill membranes as long as the fish moved forward because the flow would not be intermittent. Actually, this has been observed in both *Carcharias taurus* Rafinesque and *Anisotremus surinamensis* (Bloch) when swimming leisurely. Furthermore, fishes which might be expected to make excellent use of this simplified manner of breathing, such as many members of the Carangidae and Scombridae, have particularly well-developed opercular apparatus.

Respiration thus further complicates the study of the functioning of the remaining factors as the reaction of any fin or body movement is so modified that the effective thrust is the resultant of such and the respiratory effect, and as the respiration of fishes is an intermittent process it is clear that their mechanism would not be as efficient as a machine giving continuous flow. The resultant reaction would depend on whether inhalation or exhalation was taking place at the moment of a given fin or body movement. The exhalation of fishes is not to be confounded with the simple reactive jets of the so-called Syringograde animals which suck up water slowly and expel it with violence through the same or a nearby aperture, such as the Cephalopods, Medusae and certain Odonata nymphs. This notoriously inefficient method may be compared to the discredited jet propulsion systems of the past.

In the light of this evidence the question is no longer concerned with whether or not fishes in general receive a perceptible thrust from the exhaled water, but resolves itself into detailed study of the amount of its locomotor function for each particular species.

THE MAXIMUM SPEED OF FRESH-WATER FISHES

EMERSON STRINGHAM

THE question of the speed with which fish swim has elements which it would seem might make it popular, but there appear to be few recorded observations. It becomes of economic importance in connection with the effect of water power development on the fisheries. Against what velocity of water in a fishway can a fish ascend in order to go through the fishway, thus getting above the dam? Against what velocity can it swim up a tail race of a power house, where it might be injured by the water wheels?

The evidence consists, in part, of measurements of the velocity of water through which the fish swim. It may be safely inferred that a fish can swim a little, but not much, faster than the speed of the fastest water it is able to swim against.

An observer who has given no particular consideration to the matter might infer from the fact that fish go up rapid streams that they are able to swim continuously against such water velocities. The movement of the water of a stream is, however, very complex. At the surface there is a retardation by the atmosphere, and at the bed of the stream a greater retardation by the solid soil or rock, this retardation by the bed becoming very pronounced when the bed is unusually rough. Behind, and even on top of obstacles, the water may be nearly still while it is rapidly racing by on all sides. And at places there are eddies, even of substantial area, where the water at the edge of a stream flows in the opposite direction to that in the main channel. The author noticed a striking illustration of this below the Great Falls on the Caney Fork River, Tennessee, where floating objects traveled in an orbit at the edge of a raging current; simi-

larly, in a fishway at East Taunton, Massachusetts, there were places where the water doubled back, and the fish maintained themselves by directing their heads down the fishway. The United States Geological Survey have published extensively on the subject of stream flow; a paper by Pierce (1916) contains interesting data on the retardation of flow at the bed of a rapid current. These irregularities, furnishing helpful countercurrents and rest pockets, make it possible for fish to ascend streams that would otherwise stop them.

A Belgian engineer (Denil, 1909, pp. 33-34) has emphasized the distinction between a sudden bound and ordinary swimming. This distinction, in common with the generality of distinctions, must be regarded as relative rather than absolute, but none the less useful on that account. There would certainly be found all intermediate rates between the sudden bound and the slowest movement. The same authority recognized two sorts of bounds, that into the air and that through the water; with the former we are concerned only so far as it is evidence of velocity in the water. He reports having personally seen a large number of salmon force themselves by a distinct effort through several meters of water, of which careful measurement showed the mean velocity to be 5.00 meters per second. This represents a speed of slightly over 11 miles an hour.

Computations made on the basis of the height that a salmon rises above the water give a higher figure.¹ Day (1887, p. 73) concludes that six feet is probably as much as a salmon under ordinary conditions could accomplish. According to elementary mechanics the initial velocity of a body ascending vertically against gravity equals the square root of twice the product of the force of gravity into the height of the rise, or

$$v = \sqrt{2gs}.$$

Taking g as 9.8 meters, and s as 2 meters (about 6½ feet), the initial velocity would be not quite 6.3 meters a

¹ This method of attacking the problem was suggested by Mr. A. A. Doolittle.

second. In fact, the fish leave the water not vertically but at some angle to the vertical; therefore, the initial velocity would equal the product of the secant of this angle into $\sqrt{2gs}$. If the angle be taken as 45° the secant is $\sqrt{2}$. Indicating this initial velocity by v' ,

$$v' = \sqrt{2} \times \sqrt{2gs} = 2\sqrt{gs} = 8.8 +, \text{ for the assumed value of } s.$$

This gives the velocity in meters per second; the equivalent value in English measure is $19\frac{1}{2}$ miles an hour.

The same author (Day, 1887, p. 73) mentions reports of higher leaps which would give initial velocities of 10 meters a second or, if made at an angle of 45° , 14 meters a second.

For the benefit of those who observe fish jumping but have no special interest in mechanics the following rules are stated:

Let "m" be the height of the leap in meters measured from the highest point attained straight down to the water, and "f" be the same in feet, then

The initial velocity of a fish leaving the water perpendicularly equals $4.4 \sqrt{m}$ in meters per second, or $8.0 \sqrt{f}$ in feet per second.

The initial velocity of a fish leaving the water at an angle of 45° equals $6.3 \sqrt{m}$ in meters per second, or $11.3 \sqrt{f}$ in feet per second.

The initial velocity of a fish leaving the water at a smaller angle to the vertical would be intermediate in value between these two, and can be computed by the general formula hereinbefore derived.

The conclusion of Denil (1909, p. 34) as to more sustained effort by the salmon is that they may be expected to swim against a current of 3.15 meters a second for at least 14 meters. This figure is in close agreement with an opinion expressed by Napier (1914, p. R 40):

. . . it is well to point out the capabilities of the average sockeye observed under various circumstances. A vertical jump from still water, 1 foot or more deep, to running water above is certain under a height of 18 inches, and probable up to a height of 3 feet—a forward jump is generally uncertain. The fish can travel without rest for about 10 feet up a current of five miles per hour where the stream-line of that current is steady.

A passage can be effected up a shallow cataract 3 feet long and 1 foot high. Within limits, the direction and not the velocity of a current is the determining factor with regard to the difficulty of any passage. A sockeye

is apparently unable to pass, even from still water, a rectangular obstruction of 2 feet side with sharp edges whose up-stream face is flat and placed square to a current of five miles per hour or more.

It is possible, however, for a fish to pass an obstruction of similar size under the same conditions if the obstruction is waterworn and the edges are sufficiently rounded. Apparently these sockeye are able to pass conveniently up a current of five miles per hour when the force of that current is tending to hold the fish against a rock-face, if slightly irregular or against a slope of broken stone, when the angularity and size of the stones are, broadly speaking, inversely proportional.

The limiting velocity of a steady stream up which a sockeye is apparently capable of swimming lies between six and seven miles per hour, but only for very short distances, though a slight up-draft may help or hold a fish steady for a few moments.

A French engineer (Lavollée, 1902, p. 289) found that salmon of ordinary size appeared to find the limit of their strength in a current with a mean velocity of about 3 meters a second. In the opinion of von Bayer (1910, p. 1044) fishways, of the pool and fall or counter current types, should have a current velocity not exceeding 10 feet per second.

During a discussion of this subject at the Biological Society of Washington (D. C.) on January 26, 1918, Mr. Vernon Bailey told of spearing pike when he was a boy, and that he was able to run as fast as the pike could swim. A boy could probably run 8 or 10 miles an hour under these circumstances. Another member of the audience told of similar experiences with black bass and eels.

In the spring of 1917 the writer had an opportunity to study several fishways in Massachusetts, and to make some observations on the velocity of water up which the fish swim. These fish were *Pomolobus pseudoharengus* (Wilson), one of the common alewives. The instrument used to measure the velocity of the water was a Price penta-head meter, belonging to and calibrated by the United States Bureau of Standards. The table accompanying it showed the number of revolutions for velocities up to nine feet per second, but some of the measurements ran considerably above this. To get an approximate idea of what these velocities were the data supplied were plotted on cross-section paper, and a curve drawn through the points and extended freehand. For this rea-

son observations on velocities of more than nine feet per second are less dependable than the lower ones.

Of the places visited three yielded observations bearing on the question under consideration. At East Taunton the fish were freely using a small fishway, at least 180 of them passing out at the upper end in the course of half an hour. Measurements were made of the rate of flow at seven points in the fishway where the current appeared to be greatest, and it was found to vary from four to five feet per second. At Middleboro the fish were, on the day of observation, unable to ascend a little sloping falls where the velocity was about 11 feet per second. Just below they were swimming through one place where the current was 5.3 feet per second. At East Wareham the head of water, and therefore the velocity, could be varied. The fish swam up a slope about three feet long where the water was going down at rates of 6.1, 7.8 and even 9.8 feet per second. They were perfectly helpless when it was raised to $13\frac{1}{2}$ feet per second. At one place where the water was very shallow they failed to get up against a velocity of 9.3 feet per second. While this species sometimes jumps at least part way out of the water, they swam through the velocities here mentioned.

The indicated velocities for swimming, as distinguished from bounding, may be included in one table as follows:

SWIMMING SPEEDS IN MILES PER HOUR

<i>Lavollée</i> salmon 6¾	<i>Denil</i> salmon 7	<i>Bayer</i> 6.8	<i>Napier</i> salmon 6 to 7	<i>Bailey</i> pike 8 to 10	<i>Stringham</i> alewife 6.8
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These figures are in good accord for the medium-sized freshwater fishes, except in the case of the pike. The speed noted for the pike is perhaps to be regarded as that of a bound or jump, rather than that of swimming. For the alewife, however, the speed of 6.8 miles per hour was also for very short distances and is, perhaps, the bounding rather than the swimming speed.

The large marine fishes and mammals present a wholly different problem. It has been found by different observers (Hecht, 1916) that for eleven species of fish the

weight is equal to the cube of the length multiplied by a constant, the constant varying with species and season. If, now, the motor energy increases in general with the weight, it is quite possible that the locomotive force is a function of the third power of the length, while the resistance to be overcome increases only as the second power. Should this be the case it would explain why the speed of sharks and porpoises, observed from steamers, is so much greater than that of the little fishes of the rivers. If, on the other hand, fish like the mackerel attain these high velocities, other factors must enter.

For the medium-sized fresh-water fishes the maximum swimming speed appears to be about seven miles an hour, with the possibility of a bound at nearly three times this rate. More detailed observations will be needed to show variations with species, sex, water temperature, physiological conditions and so on.

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DO ANESTHETIZED BEES LOSE THEIR MEMORY?¹

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THERE seems to be a widespread belief that hivebees, unlike vertebrates, lose their memory on being anesthetized. Von Buttel-Reepen,² one of the foremost German bee students (p. 163), has the following to say concerning this question: "Betäubt man Bienen mit Salpeterdämpfen, Äther, Chloroform usw., so verlieren sie ihr Ortsgedächtnis. Während die Bienen sonst—innerhalb des gewöhnlichen Flugkreises auf einen anderen Platz versetzt—auf die alte Stelle zurückfliegen, so benehmen sich betäubt gewesene Bienen anders, sie bleiben im allgemeinen dort, wohin sie gebracht werden und fliegen nicht wieder an die alte Stelle zurück, sie vergessen das frühere Heim." A similar view is expressed by Dr. F. F. Phillips,³ of the United States Department of Agriculture, in his discussion of memory in the hivebee. Dr. Phillips (pp. 179-180) says: "The best evidence of memory is found in the fact that memory is sometimes lost. If bees are stupefied by tobacco smoke, by the smoke of the puff ball (an old practice) or by some anesthetic, they are unable to return to their old location and must re-orient themselves after they revive."

During the latter part of the summer of 1922, Dr. W. M. Wheeler, of the Bussey Institution, suggested that the writer anesthetize a colony of bumblebees in order to see whether they are affected in the same way as hivebees. On September 11, 1922, a colony of *Bremus* (*Bombus*) *impatiens*, consisting of the old queen, 18 young queens,

¹ Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University. No. 228.

² Das Leben und Wesen der Bienen. Braunschweig, 1915.

³ Beekeeping. The Macmillan Company, New York, 1921.

about 200 workers and a considerable quantity of brood, was discovered in the Arnold Arboretum, about half a mile from the grounds of the Bussey Institution, and this furnished an excellent opportunity for carrying out Dr. Wheeler's suggestion.

EXPERIMENT I

In the forenoon of September 12, 1922, all bees present in and about the above-mentioned nest were captured and placed in a glass jar, whereupon both bees and comb—the latter in a separate receptacle—were taken to the Entomological Laboratory of the Bussey Institution, where 100 workers and 5 young queens were etherized and marked (on the dorsal side of the thorax) with white ink. At 11 a. m., both the etherized and the non-etherized members of the colony were taken to the Bussey Bee-yard and transferred to an open observation-box containing their comb. When the old nest-site in the Arnold Arboretum was visited about 1.30 p. m., a large number of workers, including many marked individuals and two of the five marked queens, were found on or near the nesting-material which had been left in the nest-cavity.

EXPERIMENT II

About half an hour later, twenty of the marked workers which had returned to the old nest-site were recaptured and taken to the laboratory where they were again etherized and marked—this time on the dorsal side of the abdomen—whereupon they were returned to their comb in the bee-yard. When the old nest-site was visited at about 5 p. m. that afternoon, six⁴ of these twenty marked workers were again found on or near the nesting-material.

The results of these two experiments showed clearly that etherized bumblebees do not lose their memory and that etherization has a similar effect upon them as upon vertebrates. With this fact established, it seemed strange

⁴ As in the preceding case, several of the etherized bees failed to revive

that anesthetics should have a different effect on hive-bees, and the writer, therefore, decided to test the correctness of the two statements which were quoted at the beginning of this paper. In order to make this test, it was necessary to secure the services of a second person, and the writer here wishes to avail himself of the opportunity to thank Messrs. Albert and Theodore Mangelsdorf for their generous assistance in connection with the following experiments.

EXPERIMENT III

On July 8, 1923, at about 4 p. m., twelve drones and twelve workers (field bees) of *Apis mellifica* were captured in front of hive No. 1 and etherized, whereupon they were marked with white ink. After most of them had revived, they were transferred to a place about one tenth of a mile from their hive and liberated by Mr. Albert Mangelsdorf, while the writer was stationed at the hive entrance. The results of this experiment are shown in Table I.

TABLE I

Number of bees departing from place of libera- tion. ⁵			Time	Number of mark- ed bees entering hive.		
	♂	♀			♂	♀
2	1	1	5.21 P. M.	0	0	0
2	0	2	5.22 " "	0	0	0
0	0	0	5.23 " "	1	1	0
0	0	0	5.24 " "	1	0	1
0	0	0	5.29 " "	1	0	1
1	0	1	5.30 " "	0	0	0

At 5.29 p. m., one of the two marked workers which had returned, again left the hive and it therefore seemed best to discontinue the experiment.

EXPERIMENT IV

This experiment was carried out in the early part of the afternoon of August 5, 1923. Because of difficulties

⁵ At 8.30 P. M., about half of the bees used in this experiment were still crawling about aimlessly near the place at which they were liberated. They were still so dazed that they were unable to fly even when thrown up into the air.

in capturing field bees, thirteen workers which were guarding the entrance of hive No. 2 were etherized. In this state they were taken to a place about one tenth of a mile from their hive, where they were marked and placed in a box containing freshly-gathered honey. After most of the bees had revived, they were transferred to a shady place about one eighth of a mile from the bee-yard and liberated at 2.20 p. m. by Mr. Theodore Mangelsdorf, while the writer was again stationed near the hive entrance. By 2.44 p. m., all the bees but one (this one failed to revive) had departed, but up to 2.45 p. m. none of them, so far as could be determined,⁶ had returned to the hive. Professor Z. P. Metcalf, of North Carolina State College, who was watching the progress of this experiment, examined several of the bees used and found them to be young individuals which had probably never left the hive before, a fact which no doubt chiefly accounts for the negative results of this experiment.

EXPERIMENT V

On the same day (August 5, 1923) at about 3 p. m., twenty-five field bees were captured in front of hive No. 3 and etherized. In this state they were taken to the laboratory where they were marked with white ink and placed in a box containing a small piece of comb honey. About two hours later, they were transferred to the Bussey Dormitory, about one eighth of a mile from their hive, and liberated from a second story window by Mr. Theodore Mangelsdorf, while the writer, as in the two preceding experiments, was again stationed at the hive entrance, this time provided with veil and gloves. The results of this experiment are shown in Table II.

⁶ The writer had failed to provide himself with a veil, and the capture of bees in front of their hive had so enraged certain members of the colony that they resented his presence with telling effect. It is possible, therefore, that the return of some of the marked bees may have been overlooked.

TABLE II

Number of bees departing from place of liberation.	Time	Number of marked bees entering hive.
7	5.20 P. M.	0
2	5.21 " "	0
2	5.22 " "	1
1	5.23 " "	0
3	5.24 " "	1
2	5.25 " "	1
0	5.26 " "	4
1	5.27 " "	1
0	5.28 " "	1
0	5.29 " "	0
1	5.30 " "	1
0	5.31 " "	1

At 7.45 on the following morning, two of the bees used in this experiment were still crawling about in a dazed condition near the window from which they had been liberated, showing that bees, like human beings, exhibit great individual variation in their resistance to anesthetics.

The results of the foregoing experiments seem to warrant the following conclusions:

(1) Neither bumblebees nor hivebees lose their memory on being etherized, and hence are able to return to their nests or hives, provided they are in good physical condition and have not been kept under ether too long.

(2) The statements of von Buttel-Reepen and Dr. E. F. Phillips are probably based on experiments in which many of the bees were killed⁷ by the anesthetic, or so enfeebled that they died prematurely, while others were perhaps unable to fly for a considerable period as a result of the ordeal.

These conclusions also seem to be supported by the behavior of the pomace-fly (*Drosophila melanogaster*). As every geneticist knows, this insect retains its instincts (inherited memory) even after repeated etherization.

⁷ This fact may be easily overlooked, if a colony of bees is anesthetized in the hive.

A CASE OF COMPLETE SEX-REVERSAL IN THE ADULT PIGEON

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I

INVESTIGATIONS conducted during several years on the general problem of sex, and on the nature and control of sex in particular, have made it relatively clear and practically certain that the sex of numerous pigeons has been reversed in the earliest (gamete) or egg stage. In these cases the difficulties confronting the competent investigator do not so much lie in effecting the sex-reversal as in the two following aspects of the problem: First, in obtaining for others the kind and amount of crucial data for each of a large number of cases of complete reversal, and for other numbers of less complete reversal effected by the same means. And second—since the reversals are made in both directions—to find, isolate or measure the common element involved in the change of female gametes to male zygotes, and of male gametes to female zygotes. These two tasks, we believe, now face more than their inherent difficulties because of some widely accepted assumptions concerning sex which seem largely to partake of the value of facts in the minds of many geneticists—particularly among many leaders on the zoological side of this new science.

In contrast with the relatively large numbers of experimentally induced cases of sex-reversal obtainable in the egg-stage, we can expect only small numbers of proved cases of “naturally” occurring complete sex-reversal in adult birds. This follows from the already evident circumstance that this type of reversal will probably occur only in those adults which develop special diseases in particular or restricted organs. And within this selected

group it is only in those individuals which check the disease, return for at least a short period to a fair state of health after the injury or destruction of the gonad, and also further escape destruction at the hands of their owner during this long period of "uselessness," that complete sex-reversal in the adult can be accomplished.

Not many such highly selected individuals, with the requisite well-recorded history of their sex-performance in the first sex-stage, will come under the observation of the prepared investigator. Even then the gauntlet is not yet run and the case may still fail to be recorded as sex-reversal. If the investigator should decide to kill his bird before the reversal was quite complete, or if he be quite saturated with assumptions concerning a cytological foundation for hermaphroditism in birds, he would find it practically necessary to describe the case as one of "hermaphroditism." Though proved cases for the adult must probably long remain relatively few in numbers, one apparently adequate case has just been described in the fowl, and we here wish to add another from the pigeon. Even with these two cases we may hope to break down some of the assumptions to which reference has been made, and thus later obtain a fairer field for the examination of the evidence for the more numerous sex-reversals in the egg-stage.

II

Crew¹ has recently described a case in the fowl which he rightly considers the most clear-cut instance of sex-reversal in (adult) vertebrate animals yet recorded. From the owner he obtained a Buff Orpington hen 3½ years old. According to what he evidently considers unquestionable evidence given by the owner this hen had produced eggs and had hatched young from them before ceasing to lay at slightly over three years. When first brought under the observation of Dr. Crew at 3½ years this hen was still a nearly normal hen in appearance, but

¹ Crew, F. A. E., *Proc. Roy. Soc., B*, 1923, 95, 256.

was developing a tendency to crow. During the 22 months this bird was under Crew's observation her comb notably enlarged, she developed spurs from the merest rudiments to a length of 4 and 5 cms; she assumed the plumage, copulatory and fighting behavior of a cock, produced live sperm, fertilized two eggs of a virgin Buff Orpington hen and thus became the father of two young—a male and a female. These latter "were inter-bred and their progeny are typical Buff Orpington chickens."

The autopsy of this fowl—made after this bird again became sick, though dead of accident December 29, 1922—revealed very extensive abdominal tuberculosis, in which an enormous liver (340 grams), the gizzard, intestine, peritoneum and ovary were involved. The oviduct had almost disappeared and two vasa deferentia were present. Two testes ($3\frac{1}{2} \times 2$ cm) with even outlines and surfaces were found. A complete account of the histology of the nearly destroyed ovary and of the two testes has been separately given by Fell.² Crew discusses the condition of this bird after she ceased laying in the following terms:

In the autumn of 1920 she began to suffer from ovarian disease, which became noticeable in January, 1921. The disease was tuberculosis of the ovary, which progressively removed the ovarian tissue and so produced the effects of pathological ovariectomy. But it would seem that this tumor growth in its effects *so altered the general metabolism* (italics are ours) of the individual that the conditions favorable to the differentiation and growth of spermatogenic tissue were created. New sex cords developed from the germinal epithelium and spermatogenic tissue was differentiated both in the left gonad and also in the incompletely atrophied right.

Among the seven additional cases of more or less masculinized hens described by Crew there is one additional case in which the histology of the gonads revealed both ovarian and testicular tissue and for which the former owner of the bird states that eggs were previously laid. The other six cases showed one or another admixture of ovarian-testicular tissue, but there is no previous history of egg-laying. All the eight cases are interpreted by

² Fell, H. B., *Brit. Jour. Expl. Biol.*, 1923, 1, 97.

Crew and by Fell as "hens at various stages of sex-reversal" and "in every case the development of testicular tissue was preceded by ovarian atrophy or disease." It may be further noted that there can be little doubt that most of the cases of so-called "hermaphrodites" described in birds by Tichomiroff,³ Brandt,⁴ Shattock and Seligman,⁵ and several more recent observers, were in reality cases of incomplete sex-reversal. Also, that in spontaneous reversal from female to male the development of testicular tissue frequently, perhaps always, follows a tuberculous infection and destruction of the ovary.

III

The facts and details of the case observed by us may next be presented. On January 15, 1914, three pairs of presumably healthy blond ring doves were obtained from Mr. John N. Johnson, who had reared them and a few other birds at the Station for Experimental Evolution in addition to his duties as caretaker of animals at the laboratory. One pair of these birds bore leg-bands, and it thus fortunately happened that the two birds of interest to this presentation were provided with numbers wholly unlike all other numbers utilized before or since in our own breeding work. These numbers were: 16,588 and 16,580. It is not possible that either of these numbers was misread at autopsy. These two birds were said to be adult when obtained and were probably the parents of the pair of immature young obtained along with them; but this point is immaterial, since the tabulated data show that other eggs were laid 12 days after the pair was in our own care.

These two birds were given a pen separate from all other birds on January 15, 1914. Eleven eggs were produced within the following 90 days. These birds were believed to be pure blond rings, and it was decided to study the yolk size of all eggs laid. The hour of laying

³ Tichomiroff, A., *Proc. Nat. Hist Soc., Moscow*, 1887, 52.

⁴ Brandt, A., *Zeit. f. Wiss. Zool.*, 1889, 48, 101.

⁵ Shattock and Seligman, *Trans. Path. Soc. Lond.*, 1906, 57, 69.

was recorded for every egg laid and No. 16,580 was identified as the layer of these eggs. The remainder of the recorded history of this female is given in Table 1, and the remarkable record of her body weight and of its changes during a three-year period are given in the curves of Figure 1.

The data of the table leave no doubt that ♀ 16,580 laid eggs during three months at the beginning of 1914. Also that at this time this bird was not able to produce a pair of eggs within less than 9 days following the last egg of a preceding pair. This is a strong indication that the ovary of this bird was not then in a vigorous state; for long experience has shown that when the ovary of birds of this species does not lag in starting a new pair of eggs on their last growth cycle the next following egg is laid at 7 days after the previous clutch. An ovarian lag of 2-15 days was shown in each of the five instances tested. A further indication that something was wrong in the ovary of this bird, even at the time of laying this series of eggs, is found in the abnormal size difference of the two yolks of pairs D and F.

After the eggs of April 13-15 were laid it was necessary to give to this pair the eggs of other birds to incubate and rear. The record shows that these eggs were hatched and the young deserted within a week. Twenty-five days after these young were removed the female took her nest as usual for laying, but no eggs appeared! She and her male mate had manifestly gone through the time, copulations, etc., necessary for the production of eggs and both incubated normally; they were given eggs of other birds and successfully hatched and reared these young. This performance was later twice repeated during 1914, and from April to September of the following year was several times repeated. These failures to produce eggs point definitely to disease of ovary or of oviduct, or of their disappearance, during these periods.

Prior to February 1, 1915, it was realized that although these birds were vigorous and continued to copulate they

had for long produced no eggs. They were therefore brought into another building, where studies on sex behavior were being conducted daily, and so placed that we might more often observe the activities of this lag-gard pair. It soon developed that this female was usually and frequently forcing her *male* mate to function as a female in copulation. This was 10½ months after the female had laid her last egg. The pair continued to incubate without producing eggs, and 18 months after her last egg No. 16,580 was found to have developed the coo or crow of a male.

On May 28, 1916, after 6 weeks of complete idleness, this pair was removed to another building and placed with a small group of other spent and worthless ring doves. Six weeks later the emaciated male died and the weights and measurements of his testes were obtained. It was 17½ months after the death of the male, and 44½ months after the time of the laying of her last egg, that No. 16,580 died with very advanced tuberculosis.

The death occurred and the autopsy was made on December 29, 1917—just 6 years ago. On the evening of this same day the writer, after personally making the autopsy, left his laboratory to attend the annual meetings of the scientific societies then being held in Pittsburgh. Only upon his return, when it was too late to recover the discarded testes for sectioning, was it learned that this autopsy was that of the former female—not that of the *male* as supposed at the hour of autopsy—of this pair of birds. Failure to photograph or to section these testes, and thus make it possible to give to others this evidence of the final maleness of the bird, does not affect the conclusiveness of the demonstration of a complete sex-reversal. To one at all familiar with the gonads of pigeons the confusion of testis and ovary is incredible. We have indeed met with mixed and doubtful cases, but these have all been questioned and generally preserved for microscopic study.

The lack of photographs or of sections of these par-

ticular testes nevertheless persuaded us to reserve this case for presentation in connection with a complete account of our sex studies. It was thought that the reader would then have better opportunity to judge of the detail and accuracy of our work in general and thus be brought to share our conviction of the impossibility of error in this case. Crew's publication, however, now fully covers the gonad histology of a quite parallel case in the fowl, and further clears up much concerning the place and method of origin of testicular tissue within diseased and disappearing ovary. Again, our own case usefully supplements that of the fowl, since we obtained accurate data on the egg-laying history, while this part of the record for Crew's case is at least differently covered—by the credible word of the former owner, by the earliest competent records on external appearance, by the succession of changes in appearance, and by the final identification of disappearing ovarian tissue.

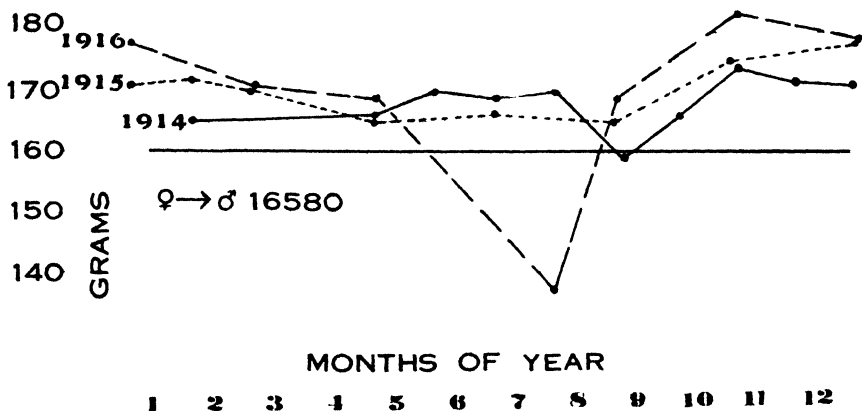


FIG. 1. Curves showing body weight of ♀ → ♂ 16,580—1914 to January, 1917.

The curves for body weight, formed from weights of No. 16,580 taken at various intervals between February 3, 1914, and January 5, 1917, are worthy of examination. During neither of three years covered by the data, do the seasonal fluctuations of the body weight approach the normal. During 1914 her weight either increased or remained high from May to August. This is the period

during which this bird lost her capacity to produce eggs; and it is the season during which body weight normally shows a decrease, attaining its minimum in September (actually attained here) or October. The amount by which the autumn weight (September) is decreased from that of February and May is both too slight and of too short duration. The curve for 1915 shows that when the bird's weight should have been lowest, in September or October (when bird began to crow as a male), it was equal to or greater than that of May. The 1916 curve shows an extraordinary loss of weight (23 per cent.) from January 3 to August 3 and a still more extraordinary gain (34 per cent.) between the latter date and November 3—this increase occurring at the season when the weight normally decreases.

Other general facts recorded in the curves are also notable. The body weight is higher than that of a normal female during all three years, and was most nearly that of a normal female during the actual period of egg-laying. The winter weight, and indeed the average yearly weight, was progressively greater with each succeeding year. Excepting only the period during which eggs were laid the body weight throughout is more nearly that of a normal male (about 170 grams) than that of a normal (about 160) female. The bearing of all this on metabolic changes in this bird is obvious.

The fact that the left testis of No. 16,580 was larger than the right is important. The general significance of this condition in pigeons was reported⁶ several years ago. We then presented evidence that this is a reversal of the normal size-relation of the two testes, and "that a male which is forced to arise from a female-producing egg may (thus) show in the relative size of its gonads an approximation to the relative size of the gonads of a female." Likewise, in the present case the larger left testis reflects the earlier femininity of the bird.

⁶ Riddle, O., *Anat. Rec.*, 1918, 14, 283.

IV

The cases of complete sex-reversal in the adult pigeon and fowl are each accompanied by data which make it possible and profitable to consider the mechanisms involved in these cases of sex-reversal. In our consideration of this important aspect of the problem we are led to conclusions differing in some respects from those of Crew. To us it is obvious that a satisfactory conclusion must take cognizance not only of both these cases of adult sex-reversal, but of all other established facts of sex and the modifiability of sex. At the outset, however, it can be said that our case and all other established facts known to us agree with Crew's conclusion:

The type of sex-organization and of the reproductive functioning of the individual are not irrevocably decided by the sex-chromosome constitution. It is certain that an XY individual—a "determined female"—can produce sperms just as efficiently as it can produce ova. . . . The transformation of the sex organization of an individual, a determined male or a determined female, into that ordinarily possessed by an individual which has the alternative sex-chromosome constitution is an established fact.

My own conclusions, and their divergence from certain other of Crew's conceptions can not be presented fully here. In general, however, our views concerning the present data are quite the same as those stated in our several publications of the last twelve years—the case here described having been available to us during the last six years. It is not practicable, nowever, to give here a full discussion of all aspects of these two cases.

The history of these two special cases of sex-reversal indicate to us that the transformation of these adult female birds into males was associated with the same general principle which we believe we have fairly established in our earlier studies, namely, that those conditions or agencies which *increase the metabolism* of gamete or zygote tend to carry development in the male direction. With this principle⁷ in mind one may then

⁷ Another principle which probably applies to these cases, but which for practical reasons can not be discussed here, is the following: In birds specifically and probably in vertebrates generally the males carry the ontogenetic development of characters associated with sex to a further (phylogenetic) point than that attained in the females.

consider the evidence that tuberculosis in particular, and the destruction of the ovary in general, may effect such an increase of the metabolism.

There is of course obvious evidence that the metabolism of the tuberculous individual is increased, and quantitative data for the human are available (see Cordier).⁸ The related facts then indicate that the two described cases of complete sex-reversal—fowl and pigeon—both changed from female to male, and this change was simultaneous (or certainly nearly so) with a change from lower to higher metabolism. In these two cases, however, there was not only tuberculosis in the organism, but a tuberculous *destruction* of the *ovary*. Probably it is only in this latter case, where the defense of the ovary for its own maintenance are largely or wholly removed by disease, that complete sex-transformation can occur in adult birds.

Tuberculosis in organs other than the ovary has also been found by us to accomplish the partial masculinization of the female pigeon. For many years we have recorded case after case of female ring doves which stopped laying, ceased showing female behavior, and later attempted to copulate as *males* with their male mates. At autopsy such birds have in most cases proved to be tuberculous. Tuberculosis induces a higher level of metabolism, and in all these cases the changes are from females to or toward maleness. Metabolic level is the common factor for these, and the numerous cases of sex-reversal in the gamete stage in the pigeon. In some of our earlier studies⁹ we have specifically dealt with the applicability of this same factor to the other several known cases of modification of sex in animals. Further, during the past 12 years we have presented parts of our accumulated evidence that this same difference in metabolic level characterizes the male- and female-producing *gametes* in the pigeon. This has carried the earlier con-

⁸ Cordier, V., *Compt. Rend. Soc. Biol.*, 1923, 88, 782.

⁹ Riddle, O., *The Amer. Acad. of Med.*, 1914, 15, 265; *AMER. NAT.*, 1916, 50, 385; *Science*, N. S., 1917, 46, 19.

ception of Geddes and Thomson into quite new territory.

In another communication¹⁰ we have shown that among ring doves the ovary is much more frequently the seat of macroscopically observable tuberculosis than is the testis. There and elsewhere⁶ it was also shown that active and progressive tuberculosis in other organs is associated with atrophy of the testis. This well-established observation makes it wholly probable that after tuberculous destruction of the ovary, the bird must check the active progress of tuberculosis in the organism in order that a fully functional testis may be formed.

We have earlier noted Crew's suggestion that the destruction of the ovary by this disease "so altered the general metabolism of individuals that the conditions favorable to the differentiation and growth of spermatie tissue were created." Though he later twice refers to this "metabolic disturbance" Crew evidently does not consider this a final or sufficient description of the matter. He also attempts to apply another, and we believe demonstrably erroneous, conception. In the application of this hypothesis he apparently quite neglects the obvious metabolic change, though he earlier speaks for its existence in the case described, and though the present writer has formulated anew "the metabolic theory of sex," as noted above, and has presented much evidence of many kinds showing that changes in metabolic level are the *primary* source and cause of sex modification and control.

Crew sponsors the view that the ovary in process of destruction by disease is stimulated to a production of sex-cords late in life, and that these proliferations, precisely because of their appearing late in life instead of within the embryo, have a tendency toward the production of maleness. This hypothesis—with an attempt to introduce Goldschmidt's "timing mechanism"—seems wholly untenable in view of Allen's¹¹ recent work which indicates that even in the mammal (mouse) there occurs a cyclical proliferation of the germinal epithelium into

¹⁰ Riddle, O., *Jour. Infect. Dis.*, 1921, **29**, 544.

¹¹ Allen, E., *Amer. Jour. Anat.*, 1923, **31**, 439.

the cortex of the adult ovary at each normal oestrous period. These data, as well as considerable amounts of earlier work, indicate that such proliferations normally occur late in life in the higher vertebrates, and that they nevertheless normally produce ovarian not testicular elements. Even if such a "timing mechanism" would account for the instances cited by Crew—and we are convinced that it fails to do so—it again wholly fails to apply to sex-reversals in the gamete stage where, by different agencies, the reversals are effected at the same stage and in either direction. It fails in still other cases.

The further suggestion that the *right* testis in the sex-transformed fowl arose from an "incompletely atrophied right" ovary, is wholly improbable in the case of the dove. We think it also improbable, and a wholly unnecessary assumption, in the case of the fowl; for primordial germ cells were originally received at this area and a temporary (essentially embryonic) right ovary was early formed there in contact with the peritoneum. That same peritoneal area and the immediately subjacent cells which actually persist would seem, from the data available, to afford sufficient point and material for the derivation of the newly formed testis in both the fowl and pigeon. The removal of the ovary, together with its influence in maintaining the conditions of its own functioning and of femininity, and the onset of a disease leading to the establishment of the specific condition—increased metabolism—favorable for male production, would seem to be the essentials required for the formation of testicular tissue from the peritoneum at the site of the embryonic ovary.

If the dove described here had died or been killed at any of several different times between the laying of the last egg and a year or two thereafter, we would then almost certainly have found an ovo-testis on the left side and a testis on the right; and also a greater or less predominance of ovary in the ovo-testis depending upon how soon the bird was killed after laying. Similarly, one or

another stage of transformation of the sexual ducts would have been found. Depending upon the stage attained at the time the bird happened to be killed, and the amount of latitude offered by an inadequate previous sexual history, this same bird could have been described as any one of several kinds of "hermaphrodite." It is a great merit of Crew's work, hardly second to his demonstration of a case of complete sex-reversal, that he has also made it clear (though by inference only does he refer to the point) that many at least of the "hermaphrodites" found in higher animals have a new meaning. The present case confirms and establishes the fact.

This new meaning the "hermaphrodites" derive from their proved relationship to the transformability or reversibility of a classic case of a chromosome-determined character. In view of the demonstrations described and discussed in this paper, and in earlier work, it can scarcely be contended that there are other chromosome-determined characters of organisms which are not transformable or reversible.

In the reversibility of characters on the one hand, and in the now obvious and unquestioned normal influence of the chromosomes on the other, biology nowadays presents the two sides of the problem of individual development. Who will venture that the plastic member of the pair is the less important in phyletic development—in evolution? Thus far genetics and cytology have each adopted the chromosomes as its own; but they bestow scarcely a limping gesture upon the facts of reversibility. The advancement of our knowledge of plasticity and controllability of hereditary characteristics does require other methods and aims than those now prevalent in genetics and cytology; but studies conducted in this newer field must unfailingly utilize the facts gathered from those two branches of science—and make a heavy call upon other branches of biology, physics and chemistry besides. The field of modifiability is not only the more alluring aspect of development—it promises results

of more practical importance. Though we may not hope to take from or give to the chromosomes of mankind, the temporary transformability—not a mere modifiability—of probably all alternative genes of every human being and of every organism is a scientific possibility which awaits only the work of the investigator.¹²

V

The complete reversal of sex from female to male in an adult ring dove has been described.

In this dove and in a similar case in the fowl earlier described by Crew the ovary was destroyed by tuberculosis. The relation of this disease to the establishment of an increased metabolism and of maleness is pointed out.

Many so-called hermaphrodites of higher forms are really stages of sex-reversal.

The demonstrated over-mastering of the sex-chromosome mechanism has wide and important applications in biology.

TABLE I

RECORD OF *St. risoria* NO. 16,580, 1914–1917 (AND OF MALE MATE)

♂ *St. risoria* (16,588) from Mr. Johnson 1/15/14.
 ♀ → ♂ *St. risoria* (16,580) from Mr. Johnson 1/15/14.

A1.	1/27	7.82	wt. yolk, 1.880.
A2.	1/29	8.54	wt. yolk, 2.135 = + 13.6 per cent. larger.
B.	2/17	(6.32)	wt. yolk, 1.940 (prematurely laid).
C1.	3/7	8.30	wt. yolk, 1.810.
C2.	3/9	9.06	wt. yolk, 2.080 = + 14.9 per cent.
D1.	3/21	7.55	wt. yolk, 1.480.
D2.	3/23	9.27	wt. yolk, 2.200 = + 48.7 per cent.
E1.	4/2	7.70	wt. yolk, 1.500.
E2.	4/4	8.61	wt. yolk, 1.700 = + 13.3 per cent.
F1.	4/13	8.41	wt. yolk, 1.820.
F2.	4/15	10.45	wt. yolk, 2.256 = + 24.0 per cent.

Incubated other eggs; hatched, deserted 5/5.

5/30/14—Incubated without laying eggs; eggs given, hatched, deserted 6/21.

7/28/14—Incubated without laying eggs; eggs given, hatched, raised.

10/24/14—Incubated without laying eggs; eggs given, removed, 11/16/14.

¹² This manuscript was written for publication in *Science*. It is hence a more condensed treatment of the subject than would otherwise have been given here.

Birds brought to new building February 1, 1915.

Feb., 1915, copulations frequent—but no eggs!

2/10/15—♂ 16,588 copulated as a ♂.

2/16/15—♂ 16,588 tail pulled out catching bird.

2/28/15—♀ 16,580 copulated as a ♂.

(New tail feathers of ♂ 16,588 only 3 cm long.)

3/ 4/15—♀ 16,580 copulated as a ♂.

3/26/15—♀ 16,580 copulated as a ♂.

3/27/15—♀ 16,580 copulated as a ♂ (at 12:15).

3/27/15—♂ 16,588 copulated as a ♂ (at 12:50).

April to September these birds often noted as willing to incubate eggs, without laying. Were used to incubate temporarily several pairs of eggs, and reared one set of young.

10/ 7/15—♀ 16,580 now “cooing as a male!”

1/15/16—Pair incubated without laying; eggs given, fed young 2/3 to 2/8.

2/20/16—Copulation observed but failed to learn which functioned as ♂.

3/ 2/16—16,588 “cooed” strongly as ♂, in attitude preliminary to mounting, but tamely concluded the performance by going to the seed pan, where he fell leisurely to eating.

3/25/16—Incubated without laying (to 4/12).

April 12 to May 28 pair wholly idle, removed to a pen with other inactive ring-doves.

♂ 16,588 dead 7/16/16. *AUTOPSY*: Considerably emaciated; lungs probably not normal but no tuberculosis; likewise none in spleen or liver; testes of rather small size—the right = 0.124 g (11.9 x 5.2 mm); the left = 0.104 g (12.1 x 4.5 mm).

♀ → ♂ 16,580 dead 12/29/17. *AUTOPSY*: Very tuberculous liver and spleen; the testes small; but not of the extremely small size usually associated with the most advanced tuberculosis; right testis, 0.030 g; the left, 0.035 g.

(There was no qualification whatever at autopsy, concerning the *maleness* of this bird; no suggestion of an ovary, which if present was certainly imbedded in the tuberculous mass involving spleen and liver. Since it was not realized at the hour of autopsy that this was the former female of the pair traces of an oviduct would also have been overlooked in minimizing the handling of this highly infected bird.)

SHORTER ARTICLES AND DISCUSSION

INHERITANCE OF PROTEROGYNY IN MAIZE

MOST maize plants are proterandrous, the pollen being shed from three to five days before the first silks appear.

An exception to this general rule is found in a variety of popcorn imported from Spain in which pollen is first shed two or three days after the first silks emerge.¹ The uniformity with which the proterogynous habit was expressed in the plants of the original importation indicated that this condition would prove to be heritable. Three generations from self-pollinated proterogynous plants have been grown and the frequency distributions of the number of days from the appearance of the first silks to anthesis for the three years are remarkably uniform.

This uniform behavior made it desirable to test the inheritance of the proterogynous habit in hybrids with normal proterandrous maize. A hybrid was made with an inbred strain of Pawnee maize which had proved to be exceptionally vigorous.² The behavior of the F_1 with respect to the proterogynous habit was not measured other than to record that the plants were proterandrous by the usual number of days and were remarkably uniform.

The F_2 and both parents were planted and the number of days from planting to anthesis, and to the appearance of the first silks as well as the number of days from pollen to silk were recorded.

The parents behaved as in former generations. The proterogynous strain averaged $2.96 \pm .18$ days from silking to pollen while the proterandrous strain shed pollen $2.3 \pm .11$ days before the first silks appeared. No proterogynous plants were found in the proterandrous strain which with respect of this character was remarkably uniform. On the other hand the proterogynous strain as in former generations produced several proterandrous plants although the two parents did not overlap in this respect. In addition to the proterandrous plants and similar also to former generations, the proterogynous strain produced several

¹ Collins, G. N. A Variety of Maize with Silks Maturing Before the Tassels. U. S. D. A. Bur. of Plant Industry Circular 107, 11 p. Feb. 7, 1913.

² Collins, G. N. Intolerance of Maize to Self-fertilization. *Jour. Wash. Acad. of Sciences.* pp. 309-312, Vol. 9, No. 11, June 4, 1919.

plants that failed to extrude the anthers and never shed pollen.

The plants of the F_2 extended the range of proterandry by ten days, two plants shedding pollen twenty days before the first silks appeared, while one plant was proterogynous by a period of thirteen days. The mean of the F_2 population, however, was proterandrous by $4.45 \pm .23$ days, while the mean of the Pawnee parent was $2.30 \pm .11$ and that of the Granada was $-2.96 \pm .18$ days. Although the mean proterandry was greater in the F_2 than in the Pawnee parent the mode was almost exactly intermediate between that of the two parents, the mode of the proterogynous parent being at -4 to -5 days, that of the proterandrous parent at 7 to 8 days, and the F_2 at 1 to 4 days. The frequency distributions for the two parents and the F_2 are shown in Fig. 1, and the means are given in the following table:

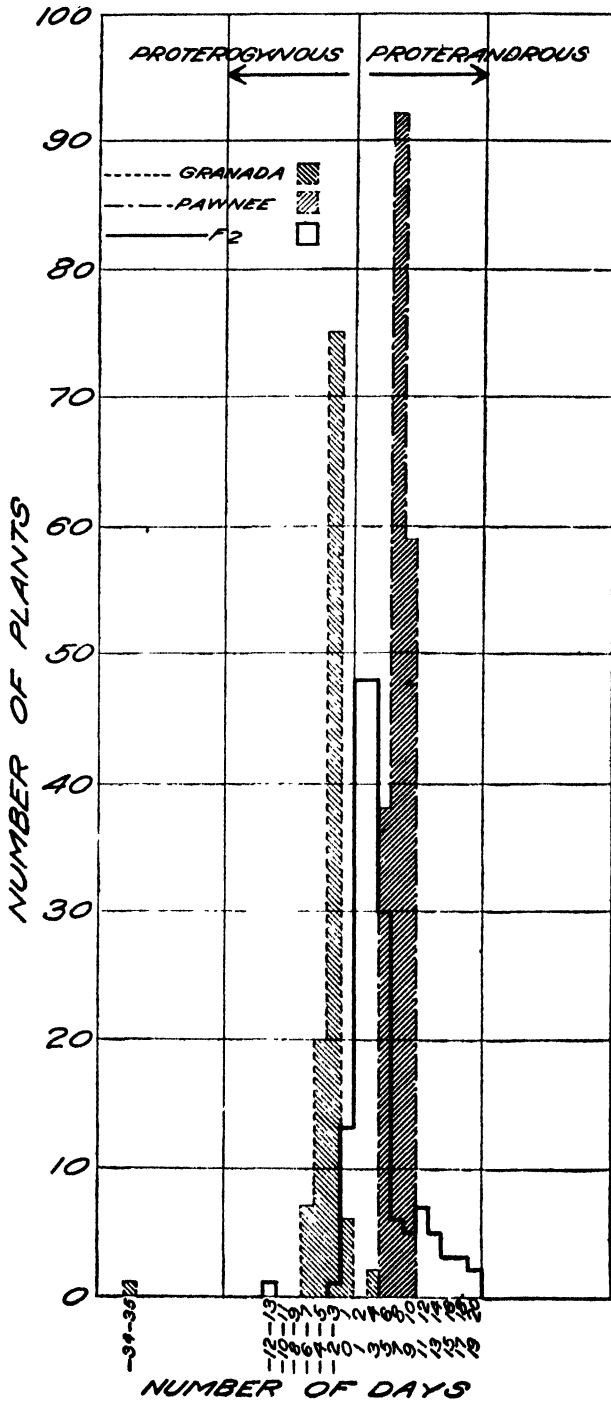
	Days to Pollen	Days to Silk	Days Pollen to Silk.
Granada	$90.6 \pm .22$	$87.6 \pm .26$	$-2.96 \pm .18$
Pawnee	$80.4 \pm .17$	$82.5 \pm .17$	$2.30 \pm .11$
F_2	$77.5 \pm .19$	$81.5 \pm .24$	$4.45 \pm .23$

The increase in the mean proterandry of the F_2 plants was brought about by a shortening of the period from planting to anthesis, while the days from planting to silking was essentially the same as that for the Pawnee parent. This fact would seem to indicate that the proterogynous nature of the pop corn parent was the result of abnormal behavior in the development of the staminate inflorescence rather than a change in the relation of the staminate and pistillate inflorescences.

Further support for this hypothesis is received from the occurrence of plants which fail to extrude anthers and the proterogyny in this case may be considered as the result of a variable expression of a male sterile condition in which pollen formation is inhibited or possibly greatly delayed, one plant having shed pollen 35 days after the first silks appeared.

The sterility in this case, however, differs from a male sterile condition often encountered in maize in which the spikelets function normally, extruding the anthers, but the anthers of such plants contain only undeveloped pollen and never dehisce. This latter type of sterility seems to behave as a simple Mendelian character recessive to the normal condition and may be identical with that reported by Eyster,³ though his illustrations and de-

³ Eyster, Lewis A. Heritable Characters of Maize VII Male Sterile. *Jour. of Heredity*, Vol. XII, No. 3, pp. 138-141, March, 1921.



scription more nearly fit the sterile plants of the proterogynous Granada pop corn.

In the Granada strain, however, the percentage of sterile plants is much less than the expected 25, the observed percentage being 13.9 ± 1.8 . This percentage of sterile plants, low from the standpoint of a simple Mendelian character, is encountered also in the F_2 population of the cross between the proterogynous and proterandrous varieties, the percentage of sterile plants in this population being 16.0 ± 1.73 . The low percentage hardly can be the result of a high death rate for plants of this genetic constitution, an explanation which so often is applicable with other recessive characters but may be brought about through the interaction of modifying factors which tend to inhibit sterility.

In this connection it is interesting to observe that the percentage of sterile plants is essentially the same in the F_2 of the cross between Granada and Pawnee as it is in the Granada parent, indicating that if modifying factors for sterility are involved in the Granada strain the same factors are present also in the Pawnee variety.

The percentage of proterogynous plants in the F_2 eliminating those plants which failed to shed pollen is 8.7 ± 1.4 suggesting the 6.25 per cent. of a dihybrid character, but an inspection of the frequency distributions show that the nature of the inheritance is not so simple.

The proterogynous plants of the F_2 have a mean of but 1.47 as compared with the mean of 2.96 days for the proterogynous parent offering little support for the hypothesis that only two factors are involved in the expression of this character and that the proterogynous tendency has been recovered completely in this hybrid. In fact, from the behavior of the F_2 plants it would seem that an extreme condition of proterandry could be established with more certainty from this hybrid than could an extreme condition of proterogyny, though it is clear that the proterogynous tendency is inherited and can be transmitted through hybrids with the normal form.

The nature of the causes underlying the expression of the proterogynous condition are obscure. Photoperiodism, which plays such an important part in the development of the floral organs, does not seem to be an important factor in determining the relative maturity of the sexes in maize. While it had been possible by the use of an extremely short day to cause the development of pistillate flowers, or even apogamy with all the

intermediate stages, in inflorescences which under normal conditions would be entirely staminate, we have not produced artificially a condition approaching the sterility manifested in the proterogynous Granada strain.

Proterogyny in maize may be considered as a reversion to an ancestral condition since it is normal to *Tripsacum*, *Euchlaena* and *Coix*. It must be noted, however, that with the advancing season the inflorescences of *Tripsacum* change gradually from a proterogynous to a proterandrous condition, a change accompanied by an alteration in the ratio of staminate to pistillate spikelets, the late plants having a higher percentage of pistillate spikelets than the early ones. This behavior certainly would seem to indicate that the relative maturity of the sexes in this genus was subject to environmental influences and the phenomenon is strongly suggestive of photoperiodism.

No such behavior, however, has been found in *Euchlaena* and even under artificial long and short days if staminate spikelets are developed at all the inflorescences always are proterogynous. In this connection, however, it should be noted that the relative maturity of the two sexes might depend upon a particular combination of daylight and darkness, a combination which was not used in the experiments tried thus far.

The nature of the inheritance of proterogyny in the Granada-Pawnee hybrids is very similar to that encountered in crosses between maize and teosinte. In the teosinte-maize hybrid, however, the proterogynous condition is not recovered invariably, some hybrids producing only proterandrous plants.

In teosinte and teosinte-maize hybrids also no indications of a male sterile condition have been found, all plants shedding pollen even though extremely proterogynous.

SUMMARY

Proterogyny, which is the normal condition in *Tripsacum*, *Euchlaena* and *Coix*, has been found to be normal also in a variety of maize from Spain. In hybrids with normal proterandrous maize the proterogynous condition behaves as a recessive character and the F_1 is proterandrous.

Proterogynous plants are recovered in the F_2 , though in too few numbers for a simple Mendelian character. The frequency distribution of the F_2 plants of the maize hybrid is very similar to those obtained in teosinte-maize hybrids. From the occurrence of male sterile plants, and the character of the frequency distri-

bution, it seems probable that proterogyny in maize is the result of a variable expression of a male sterile condition, the variability being brought about through the interaction of modifying factors.

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OBSERVATIONS ON A NEW TYPE OF WINTER FEED- ING GROUND FOR THE FRINGILLIDAE

WHILE conducting some investigations of the animal micro-organisms in the sprinkling filter of a sewage disposal plant at Bound Brook, New Jersey, the writer, during the months of December, January and February, had several times observed large flocks of birds apparently busily engaged in securing food from the surface stones of the filter bed, working rapidly between the periods of the activity of the sprays. A closer study of these birds and of the sort of food they were securing from the filter bed brought to light some new and interesting relationships.

At the Bound Brook Sewage-Disposal Plant, after the water from the incoming sewage has had its suspended materials partly removed in settling tanks, it is sprayed from a multitude of nozzles over a bed six feet in depth, composed of irregular basaltic stones, about the size of small eggs, to insure purification before it is allowed to drain off into a neighboring brook.



FIG. 1

A portion of the filter bed, showing the sprays in operation. In winter the beds are often covered with snow and ice except in the areas reached by the sprays

Fig. 1 shows approximately one third of the bed. On the stones over the surface of this bed there flourishes a rich greenish mat, composed of *Oscillatoria* and other algae, and underneath the stones, and continuing to the bottom of the bed there collects a heavy, slimy growth consisting of various fungi, with entangled bacteria in gelatinous matrix, cellulose débris, protozoa, nematode and annelid worms, tardigrades, rotifers and other minute forms. Near the surface of the bed there develop immense numbers¹ of moth flies (*Psychoda alternata*), the larvae and pupae of which are found together throughout the winter, just beneath or on the sides of the surface stones, together with many newly emerged adults (Figs. 2, 3 and 4). This transformation is continually taking place even during the very coldest months of winter. It was these forms that the birds observed were chiefly engaged in gathering. A few of the larger annelids found in the filter film (covering the stones), such as *Pristina* and *Aeolosoma*, are present near the surface, but usually underneath the surface of the film, and invisible.

The birds feeding on the filter bed were the tree sparrow (*Spizella monticola*), song sparrow (*Melospiza melodia*), junco (*Junco hyemalis*) and the goldfinch (*Astragalinus tristis*). These occurred in flocks of from 50 to 150 individuals, with the juncos and song sparrows the most numerous, and the tree sparrows and goldfinches in less numbers. At times there were two or more separate flocks feeding on different portions of the filter bed, making a total of some 300 or more individuals at work. From observations on the rapidity of feeding it was judged that, on an average, each bird secured one bit of food (doubtless one larva, pupa or adult *Psychoda* fly) every two seconds, making 30 individual organisms per minute for each bird, or 1,800 per hour. This would make a total of 270,000 organisms for a flock of 150 birds, or over half a million when the flocks totaled 300, as they sometimes did. And if 150 birds, at least, were at work on the beds during, let us say, four hours a day, the number of organisms consumed would reach 1,080,000.

The birds fed upon the filter bed during two-minute periods when the sprays were quiet, flying to other parts of the bed when

¹ The average number of *Psychoda* larvae, pupae and adults for ten stones from various portions of the surface of the filter bed taken during the months of January and February was 54. Just beneath these stones were almost solid masses of larvae, which were continually crawling towards the surface along the sides of the surface stones, and there pupating and emerging.

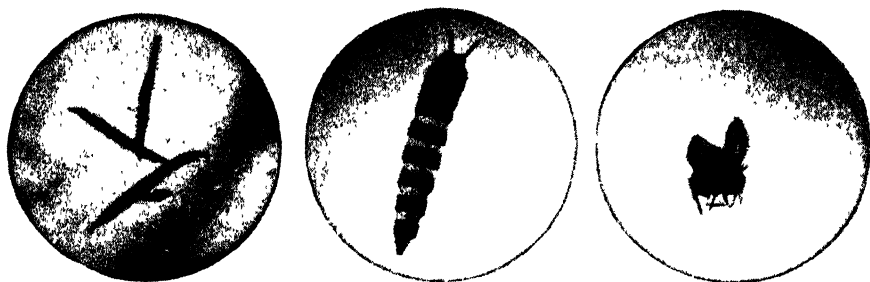


FIG. 2. Larvae of the *Psychoda* fly (\times cir. 10). FIG. 3. Pupa of the same (\times cir. 18). FIG. 4. Adult (\times cir. 10).

the sprays over the portion where they were feeding began operation. Frequently, during the active period of the sprays they left the bed altogether and flew into nearby bushes and weed patches, to continue their feeding upon weed seeds. Not all the birds left the beds, however, even during the spraying. Some song sparrows and occasionally some juncos were seen hopping about under the sprays, avoiding the descending water-drops as best they could.

It is interesting to note that these birds, typical seed-eaters, neglected the weed patches, of which there were many close to two sides of the filter bed, in preference for the *Psychoda* larvae, pupae and adults. Within a stone's throw from the filter bed, on two sides, were patches of bushes and woodland wherein grew quantities of such weeds as the giant ragweed, smartweed, pig-weed, moth mullein, evening primrose and others. An examination of these weeds showed that they were plentifully supplied with seeds. A microscopic examination of film from the surface of the filter bed revealed few or no weed seeds.

Of the birds seen on the filter bed the tree sparrow is probably the most exclusively seed-eating, seeds forming about ninety-eight per cent. of its food. Fifty per cent. of this is weed seed.² The junco is also largely a seed-eater, while the song sparrow feeds upon weed seeds to the extent of about fifty per cent. The goldfinch, while chiefly a seed-eater like the rest of the fringillids, has been seen, in winter, to consume large numbers of plant lice eggs. One stomach examined contained 2,210 eggs of the white birch aphid.²

The presence of an immensely rich supply of food in the shape of *Psychoda* larvae, pupae and adults, and the ease with which

² Forbush, E. H., "Useful Birds and Their Protection," 1905.

this can be secured in winter, has resulted in a considerable modification of the dietary of those birds feeding at the filter beds. Newcomers are attracted to the beds probably both by the sight of bare ground in the midst of a snow-covered landscape, and by the presence of flocks of birds of their own or of a congenial species.

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HELIX PISANA IN CALIFORNIA

MANY years ago¹ Professor J. L. Howe published a detailed account of the Virginia colony of *Helix nemoralis*, showing that it had produced an extraordinary number of hitherto unrecorded band-variations. Another very variable species introduced from Europe is *Helix* (*Euparypha*) *pisana* Müller, which has become so great a pest at La Jolla, California, that strenuous measures have been taken to exterminate it. Thinking that the La Jolla colony might show some deviations from the usual type, I asked Mr. A. J. Basinger for specimens, and he has very kindly furnished an abundant supply. An analysis of this material shows that in form and texture it represents typical *H. pisana*, but varies in color and marking as follows:

- (1) *pisana* proper, with numerous bands. 351 shells, of which more than 20 have the banding confined to the region just before the aperture.
- (2) variety *bifrons* Moquin-Tandon, with the banding confined to the lower part of the shell, below the periphery. 122 shells.
- (3) variety *alba* Shuttleworth. Whitish, tinged with ochreous, unbanded or with slight traces of bands. 213 shells.
- (4) variety *subzonata* Bourguignat. With broad pale fulvous bands above and below the periphery, and often a more distinct basal band. 25 shells.
- (5) variety *interrupta* Moquin-Tandon. With a very slender, more or less broken (punctiform) peripheral band, and usually some more or less interrupted banding below. Seven shells.
- (6) variety *punctella* Moquin-Tandon. A more extreme form of the last, with series of dots in place of bands. Two shells.
- (7) variety *sagittifera* Taylor, with arrowhead-like markings, but in our specimen they are confined to the region just before the aperture. One shell, not adult.

Although it is thus possible to catalogue seven named forms, the series is in fact a quite ordinary one, not showing any de-

¹ AMERICAN NATURALIST, XXXII, December, 1898.

parture from the condition observable in many European localities. While we lack experimental evidence, we may infer that the banding is due to at least two factors, a banding factor proper, and an activator of this factor. The latter may not operate early in the life of the snail, so that many shells are banded only toward the aperture. The variety *alba* probably lacks the activator. In variety *subzonata* groups of linear bands are fused and represented by pale reddish zones; apparently a modification of the banding factor. In *interrupta* and *punctella* we may suppose that the activator operates at regular intervals, how or why is not known. The *sagittifera* type is even more singular, the pigment forming foci on the mantle edge widening at intervals. It is evident that the species will repay experimental investigation.

The above list of varieties gives little idea of the range of variation of this snail, which has been figured by J. W. Taylor on two beautiful colored plates.² The small island of Porto Santo, in the Madeira group, produces *H. pisana* of much greater variety, as I have myself observed.³ The variations seem to have little or nothing to do with environmental conditions, very diverse forms existing under the same environment. Taylor cites a case in which change of locality appears to have affected *H. pisana*, but it should be reinvestigated. The story is as follows:

M. Mabille has also described its introduction in 1870 to the banks of the River Marne at Charenton, near Paris, as being due to a friend who, on his return home from travelling in the south of France, brought with him a large basketful of these snails for table use, but falling ill, the nurse, who attributed his malady to the snails, emptied the basket containing them upon the river bank, by the omnibus depot, where the environment being favorable, they prospered, but, according to Dr. Germain, have gradually become modified, as though at first all were fine, strong and distinctly banded shells, the great majority are now, though still of good size, of a delicate texture and a pure subtransparent white.

Nevertheless, in spite of its wide distribution and great variability, the species appears to have produced few distinct segregates. The British colonies, of which that at Tenby is the best known, do not show any special features; but it is at least possible that the snail has been introduced into Britain by human means within historic times. It has however been known as British since 1777.

² Monograph of the Land and Freshwater Mollusca of the British Isles. Part 19. (1912.)

³ *Proc. Malacological Society*, XIV (1921), p. 196.

In France, Locard recognized five members of the *H. pisana* group: *H. pisana* Müll., *H. pisanella* Servain, *H. cuttati* Bourguignat, *H. carpiensis* Let. and Bourg., and *H. bertini* Bourguignat. Germain⁴ does not hesitate to reduce all these to *H. pisana*, which is the only *Euparypha* described in his work.

In Syria, Morocco, etc., there are some distinct species and races of *Euparypha*. One of the most remarkable is the *H. ustulata* of Lowe,⁵ confined to the Salvage or Selvage Islands, far out in the Atlantic. This is certainly a valid species. Unfortunately, it appears to be on the point of extinction. My friend Mr. A. C. de Noronha recently visited the Selvages, and informs me that whereas *H. ustulata* was formerly common, it now becomes rarer and rarer, perhaps owing to the lack of sufficient rain in recent years. He could not find a single living adult.

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⁴ Mollusques de la France et des Régions voisines. (1913.)

⁵ This species is listed by Pilsbry as *H. macandrewiana* Pfeiffer. Lowe's name is the valid one, and is not preoccupied. The name *ustulata* Jay, 1839, was published under *Bulimus*, and refers to a color-variety of *Cochlostyla chrysalidiformis* Sowerby.

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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE. X. THE DURATION OF LIFE OF DROSOPHILA MELANOGASTER IN THE COMPLETE ABSENCE OF FOOD¹

RAYMOND PEARL AND SYLVIA L. PARKER

IN the "Biology of Death" Pearl (52) advanced the idea that the inherent potentiality of the organism in respect of longevity was determined by its individual, in-born physico-chemical *organization*, using this word in the sense which has been so thoroughly developed by Henderson (65) in his "Order of Nature." It was further suggested that the actual realization or expression of the inborn potentiality of longevity was in major degree a function of the environment in which the life was lived. The environment determines in considerable part the rate at which the vital resources are used up. Since this suggestion was first made a good deal of evidence not then in hand has accumulated which indicates that the conception is a sound one (*cf.* particularly VII (57) and VIII (62) of this series).

Normally the living organ gains its energy for the conduct of life, and material for the repair, within limits, of the wastage of its tissues in the business of living, by the process of taking food. In other words the regular re-winding of the vital clock is accomplished by feeding. If this renewal or re-winding process is defective in any particular the result will be to shorten life below what

¹ Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University. No. 100.

would have been attained under more perfect metabolic functioning. But always when we study duration of life under normal conditions we are dealing with the combined effects of two variable complexes, inborn organization, on the one hand, and environment, including renewal of available energy and substance by food, on the other hand. Suppose now we eliminate the effect of as much as possible of this second complex experimentally. We shall then be in a better position to estimate how much of the normal variation in duration of life observed among different individuals is due to difference in their inborn make-up, their physico-chemical organization.

How can this be done experimentally? Clearly by doing two things: (a) preventing completely access to food of any sort, (b) keeping temperature, moisture and as many other variables of the physical environment as possible, constant throughout life. Such differences amongst individuals in respect of duration of life as then appear will be almost wholly due to innate protoplasmic, structural, constitutional differences, since the small residue of uncontrolled environmental variables will be unimportant ones, trivial in their effect upon the organism. The form of the life curve so obtained will be in a sense that which is basic to the species or variety. The idea of controlling the food factor of the environmental complex by starvation is methodologically of importance. It is extremely difficult to ensure experimentally that two different animals get identical food, quantitatively and qualitatively. But, with some care, it is easy to see that neither gets any food at all. In short, by complete starvation we can make constant the most difficult of all environmental variables to control accurately in an experiment.

These considerations suggest that such starvation life curves may furnish a powerful analytical tool for the more penetrating study of the biology of life duration. Consider specifically the case of *Drosophila*. We have shown in this laboratory (57), (62), (66) that certain genetic constitutions are invariably associated with cer-

tain definite forms of life curves. Wild type *Drosophila* has a characteristic life curve with a definite absolute mean duration of life, and with a characteristic shape. These attributes of the life curve are constant under constant environmental conditions. Under the same environmental conditions flies which carry the gene for the recessive wing character *vestigial*, whether alone and pure, or in combination with other mutant genes, exhibit life curves which differ widely in every important respect from the curves which describe the duration of life of wild type flies. They have a much shorter absolute duration of life than the wild type and the shape of the life curve when put upon a comparable basis by measuring age in centiles of the equivalent life spans (*cf.* Pearl (61)) is widely different from that for wild type flies. All this is clear. But do these differences depend upon (a) differences in the inborn physico-chemical organization, solely and *per se*, of the two sorts of flies, or upon (b) differences in the effective reactions of these two kinds of flies to the *same* environment, including most particularly food? Or, to put the experimental question, will wild type and vestigial strains of *Drosophila* show the same kind of differences in their life curves when these curves are determined under conditions of complete starvation that they do when both sorts of flies are fed the same kind of food? It is the purpose of this paper to present experimental evidence answering this question.

If the answer to this last question should turn out to be affirmative, it is evident that starvation life curves would offer a neat and time-saving method of studying life duration. For if the starvation life curve is merely a sort of foreshortened replica of the normal life curve of fed individuals, it will be clearly possible to accumulate data rapidly for many different forms. One can think of general facts of natural history that suggest that there is a correlation of this sort. The mouse is the shortest lived mammal. Its ability to endure starvation is extremely limited. In some experiments made in this

laboratory a number of years ago the longest time we were able to keep a wild mouse alive without food (but with water) was about 18 hours. This accords reasonably with the experience of others on the point. On the other hand, snakes, especially the larger ones, are known to be normally long lived. Wall (67, p. 63) states that the python in captivity will sometimes refuse food for long periods, "and without suffering perceptibly. Ferguson records one that fasted for over a year in the Trivandrum gardens, but changed its skin more than once, and always looked glossy and in perfect health." Many other observations of similar import might be cited.

There is little in the literature regarding duration of life in insects under conditions of starvation that has any particular pertinence to our present inquiry. Important data are those of the Raus (64, 68, 69) on the duration of life in saturniid moths which do not take food in the imaginal stage. In the preceding paper in this series we have prepared the l_x line, on a centile age base, calculated from their data for *Telea polyphemus*. It shows a form of curve intermediate between the wild *Drosophila*-man-*Proales* type of life curve and that characteristic of vestigial *Drosophila*. But of the greatest significance is the fact that, in the absence of food, there is in *Telea* an essentially similar amount and kind of individual variation in duration of life to what there is in normally fed individuals of other forms of life. This is shown by the similarity in form of the l_x curve for *Telea polyphemus* and for wild *Drosophila* or man, for example.

Vinokuroff (70) gives the mean duration of life of *Musca domestica* when starved without water as 1.3 days, and when starved, but given water, as 1.8 days.

Lutz (13) gives rather extensive data on the duration of life of *Drosophila* given water but not food. His data will be compared in a later section with those obtained in the present study.

Barrows (71, p. 517) incidentally mentions that *Drosophila* supplied with distilled water, but given no food will

not survive much longer than 24 hours. "If they are kept without food much longer than this, they begin to die and few survive sixty hours."

Holmes (72) studied the effect of starvation of the larvae of *Drosophila* upon the sex ratio, but gives no data on the influence of starvation upon the duration of imaginal life.

Kopec (73) has lately published the results of extensive studies on the effect of intermittent starvation of the caterpillars of *Lymantria dispar*. The moths do not take food in the imaginal stage. "Starvation of caterpillars has no distinct effect on the duration of the imaginal stage." But *total* duration of life, from hatching to death, is prolonged by the intermittent starvation because of the extension of larval life. This prolongation may amount to 25 to 30 per cent. relative to the controls. Interesting and valuable as these observations are, they are concerned with an essentially different problem than that of the present study.

Glaser (75, 76) has recently contributed some extremely interesting and valuable experimental results regarding the effect of different food conditions upon duration of life in several species of flies. He notes that in the total absence of food *Musca domestica* lives only from one to two days. The numbers dealt with in these particular starvation experiments were small (12 flies all told) and it is impossible to construct a life table on such a meager basis. In general Glaser's experiments have been directed towards quite different problems than that of starvation.

Methods

To carry out critically an experiment on duration of life under starvation involves a number of difficulties. For the particular problem here dealt with it was necessary to have a large number of flies all emerging from pupal to imaginal life at about the same time, and under such conditions that they could not possibly get any food at all as imagoes. To attain this end the flies for these

experiments were reared in glass tubes 3 cm. in diameter and 11.5 cm. long. Through the larval and pupal periods the lower end of the tube was closed with a cork stopper on which a layer of standard food (27) of the usual depth had been poured. The top was closed by a cotton plug as usual. At the end of eight days, when the parent flies were removed from the tubes, the bottom cork and attached food were also removed and replaced by a clean cork. During this eight-day period most of the larvae had crawled out of the food and pupated on the sides of the tube, just as in the ordinary breeding bottle. Of course in this way some of the progeny were lost—those which had not pupated, or which had pupated on or near the surface of the food—but since all that was desired was to obtain a large sample of flies, raised under uniform conditions, all emerging within a short interval of time and having no food in the adult state, this was no objection to the method. Pupae could, of course, have been individually removed from ordinary breeding bottles to clean dry bottles for hatching, but this method would have taken much more time, besides introducing the possibility (indeed certainty in many cases) of injuring the pupae in handling. Progeny flies were removed every six hours, etherized, and counted out into clean, empty one ounce vials, in the different density groups. Each interval's hatch was distributed proportionately among the various densities.

The next point to be considered was that of moisture. It was obviously undesirable to run the duration of life tests in an absolutely dry atmosphere, because in addition to the starvation there would then be superimposed a desiccation effect due to evaporation of water from the flies' bodies. On the other hand, it was equally undesirable to have standing water where the flies could get at it. To that there are various objections. One important one is that it is practically impossible to furnish water to flies and not have the water contain some nutritive material in solution or suspension, particularly after

the first fly has been in contact with it. It should be realized when one is working with very small animals that any experiment which states that drinking water is furnished to otherwise starved individuals is really not a starvation experiment at all, but an experiment in feeding dilute food, unless the most extraordinary and practically impossible chemical precautions are taken. The theoretically most desirable procedure would seem to be to keep the flies during the experiment in an atmosphere so humid that there would be no significant evaporation from their bodies, and at the same time not sufficiently saturated to precipitate water in the tubes so that the flies could drink it. This state of affairs would prevent any desiccation effect from complicating the results, and also keep the flies from getting minute but real amounts of food in the guise of drinking water. After some preliminary experimentation the conditions desired were realized in the following manner.

All the duration of life bottles were kept in one electric incubator maintained at 25° C. The atmosphere was kept moist by trays of wet sand placed in the bottom of the incubator. Dry and wet bulb thermometers kept in the incubator throughout indicated that the relative humidity was held practically constant at about 80 per cent. This prevented any significant desiccation of the flies, as indicated by the condition of the dead ones. At the same time no standing drops of water condensed in the tubes.

The dead flies were removed and recorded every six hours, preliminary experiments having shown the total life span to be so short that several observations daily were needed to give enough points to construct the life tables.

Six hundred and fifty tubes were all started the same day, May 3, 1923, with three pairs of flies in each—300 tubes of Old Falmouth stock, 150 of *var. v.* and 350 tubes of pure vestigial stock. The paper and food were removed May 12, 1923. The flies began emerging May 14; the tubes were all emptied at 8 A. M., May 14, and this taken

as a starting point, that is, these first flies whose age was not accurately known were discarded, but the flies were removed at 2 P. M. and at 8 P. M., May 14, and at 2 and 8 A. M. and 2 P. M., May 15. By that time over 500 flies of exactly known age had been obtained for each density group of each stock and these were taken to be large enough samples for the experiment.

For help in carrying through the six hourly observations over more than four days continuously we are indebted to Dr. Mary Gover and Mr. James Krucky.

The plan of the experiments was as follows: approximately equal numbers of each sex of two stocks as above described were run in three density series; one of five flies per one ounce vial, one of 50 flies per one ounce vial, and one of 100 per one ounce vial. Other experiments (Pearl and Parker, 49, 74) had indicated that these densities represented conditions (a) well below, (b) approximately at and (c) well above the optimal density of population for wild type *Drosophila* at 25° C.

Data

The survivorship distributions, on the basis of 1,000 flies starting together at emergence, are given in Tables I and II.

TABLE I

SURVIVORSHIP DISTRIBUTIONS OF DROSOPHILA IN ENTIRE ABSENCE OF FOOD

(in vials)	Wild Type (Line 107)						Vestigial					
	Density 5		Density 50		Density 100		Density 5		Density 50		Density 100	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
3	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
9	998	998	997	997	997	997	1,000	997	990	987	1,000	1,000
15	990	990	988	981	988	981	997	993	977	967	997	997
21	986	986	981	978	981	978	986	969	970	961	997	991
27	967	967	978	966	978	966	972	938	961	935	977	966
33	917	917	960	944	923	903	923	903	924	908	944	948
39	790	806	890	897	784	831	784	831	786	866	812	914
45	511	570	803	803	422	731	422	731	536	788	545	840
51	192	208	516	516	192	483	192	483	178	618	155	637
57	62	61	214	214	42	214	42	214	23	294	23	323
63	7	11	0	0	10	45	10	45	0	59	6	111
69	0	0	—	—	0	14	0	14	—	3	0	18
75	—	—	3	3	—	3	—	3	—	0	—	—
81	—	—	0	0	—	0	—	0	—	—	—	—
Absolute No. of flies	276	279	288	295	321	321	321	321	304	306	341	325

TABLE II

SURVIVORSHIP DISTRIBUTION OF *DROSOPHILA* IN ENTIRE ABSENCE OF FOOD.

BOTH SEXES TOGETHER

Age (in hours)	Wild (Line 107)			Vestigial		
	Density 5	Density 50	Density 100	Density 5	Density 50	Density 100
3	1,000	1,000	1,000	1,000	1,000	1,000
9	996	995	997	998	989	1,000
15	989	983	984	995	972	997
21	982	976	980	977	966	994
27	968	955	972	955	948	971
33	917	925	952	913	916	946
39	798	784	853	808	826	862
45	541	477	651	577	662	689
51	200	216	334	338	398	390
57	61	86	154	128	159	170
63	9	29	45	28	30	57
69	0	7	6	7	2	9
75	—	2	2	2	0	0
81	—	0	0	0	—	—
Absolute No. of flies	555	583	641	577	610	666

Before taking up the discussion of these distributions it is desirable to have it have the chief biometric constants of the mortality. These are given in Tables III and IV.

TABLE III

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF STARVED FLIES.

SEXES SEPARATE

Density (flies per bottle)	Wild type. Line 107					
	Mean (hours)		Standard deviation (hrs.)		Coefficient of Variation	
	♂	♀	♂	♀	♂	♀
5	44.50 ± .36	45.03 ± .36	8.77 ± .25	8.91 ± .25	19.70 ± .59	19.79 ± .59
50	42.21 ± .31	46.94 ± .44	7.69 ± .22	11.08 ± .31	18.23 ± .53	23.61 ± .69
100	44.30 ± .29	50.89 ± .40	7.64 ± .20	10.73 ± .29	17.24 ± .47	21.09 ± .59
Vestigial						
5	43.97 ± .33	48.72 ± .45	8.24 ± .23	11.31 ± .32	18.74 ± .55	23.22 ± .68
50	44.07 ± .35	50.31 ± .47	8.95 ± .24	12.42 ± .33	20.31 ± .58	24.09 ± .69
100	44.74 ± .27	52.47 ± .37	7.30 ± .19	9.98 ± .26	16.31 ± .43	19.03 ± .52

TABLE IV
BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF STARVED FLIES.
SEXES COMBINED

	Mean (hours)		Standard deviation (hrs.)		Coefficient of Variation	
	Wild type	Vestigial	Wild type	Vestigial	Wild type	Vestigial
5	44.77 \pm .25	46.36 \pm .29	8.84 \pm .18	10.19 \pm .20	19.76 \pm .42	21.97 \pm .46
50	44.60 \pm .28	47.20 \pm .30	9.85 \pm .19	11.11 \pm .21	22.08 \pm .46	23.53 \pm .48
100	47.59 \pm .26	48.51 \pm .25	9.88 \pm .19	9.53 \pm .18	20.75 \pm .41	19.64 \pm .38

Mean Duration of Life under Complete Starvation

The first outstanding result of the experiment is that *under complete starvation the duration of life is substantially the same or even longer in vestigial than it is in wild type flies.*

Considering the means of Table III we have the following system of differences:

Males. Vestigial mean—wild type mean.

Density 5; 43.97 — 44.50 = — .53 \pm .49 (1.1)

Density 50; 44.07 — 42.21 = + 1.86 \pm .47 (4.0)

Density 100; 44.74 — 44.30 = + .44 \pm .40 (1.1)

Females. Vestigial mean—wild type mean.

Density 5; 48.72 — 45.03 = + 3.69 \pm .58 (6.4)

Density 50; 50.31 — 46.94 = + 3.37 \pm .64 (5.3)

Density 100; 52.47 — 50.89 = + 1.58 \pm .55 (2.9)

Of the six differences five are positive in sign, signifying that the vestigials had a longer mean duration of life than the wild type. Three of the differences are probably not significant, while the other three probably are, as they are four or more times as large as their probable errors (the figures in parenthesis give the values of the ratio Difference/Probable Error of Difference).

Taking the mean of Table IV, where the sexes are combined in the computations we have the following differences:

Vestigials mean—wild type mean.

Density 5; 46.36 — 44.77 = + 1.59 \pm .38 (4.2)

Density 50; 47.20 — 44.60 = + 2.60 \pm .41 (6.3)

Density 100; 48.51 — 47.59 = + .92 \pm .36 (2.6)

Here the differences are all positive. We believe that the whole system of differences, having regard for the probable errors involved, is such as to warrant the conclusion that the mean duration of life under complete starvation is certainly *not shorter* in vestigial flies than in wild type flies. It is *probably* slightly longer, but we have no desire to stress this difference in the positive direction. It may be taken merely to strengthen the conclusion that the vestigials do not have a shorter life under starvation than the wild type flies.

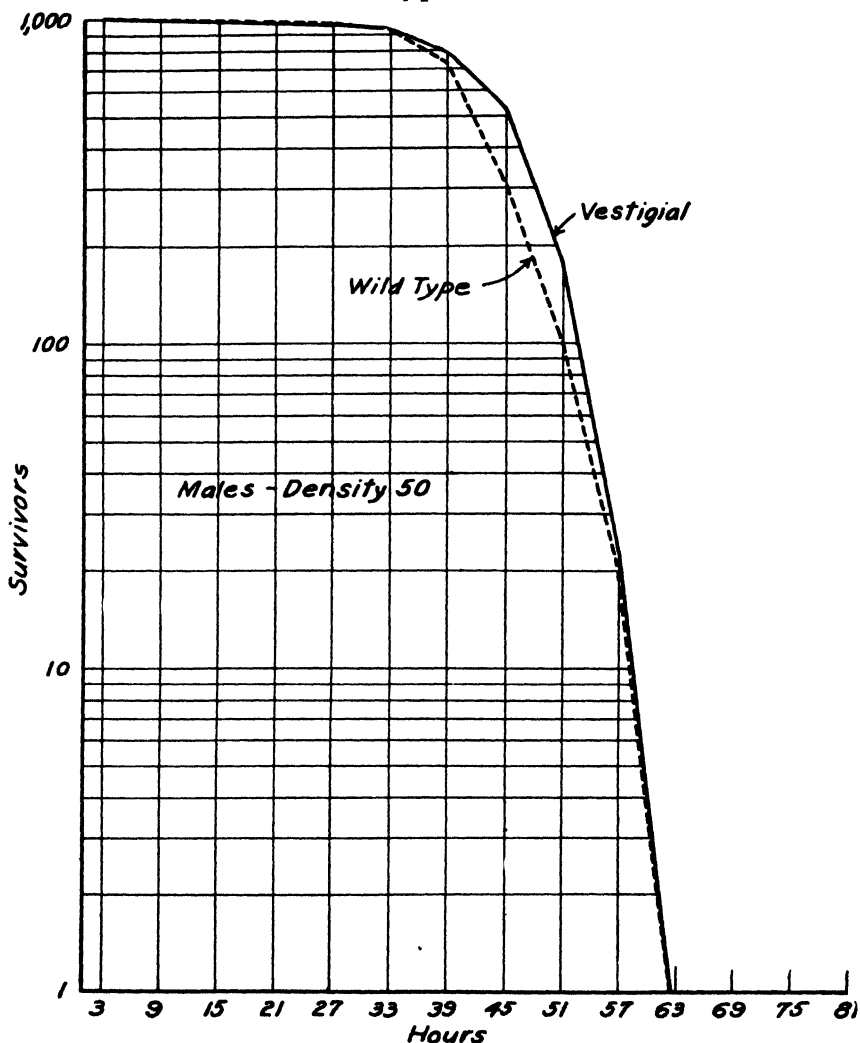


FIG. 1. Survivorship lines for wild type (broken line) and vestigial (solid line) *Drosophila* males, under complete starvation.

This result is also shown in a striking manner if we compare the survivorship lines of Tables I and II. This is done graphically in Figs. 1 to 3, inclusive. It is not necessary to show the lines for all three densities. The comparisons for one density, namely, 50, will sufficiently demonstrate this point.

It is evident that under the conditions of complete starvation that obtained in these experiments the vestigial flies lived quite as long, or a little longer, than the normal, wild type flies. This result is in marked contrast

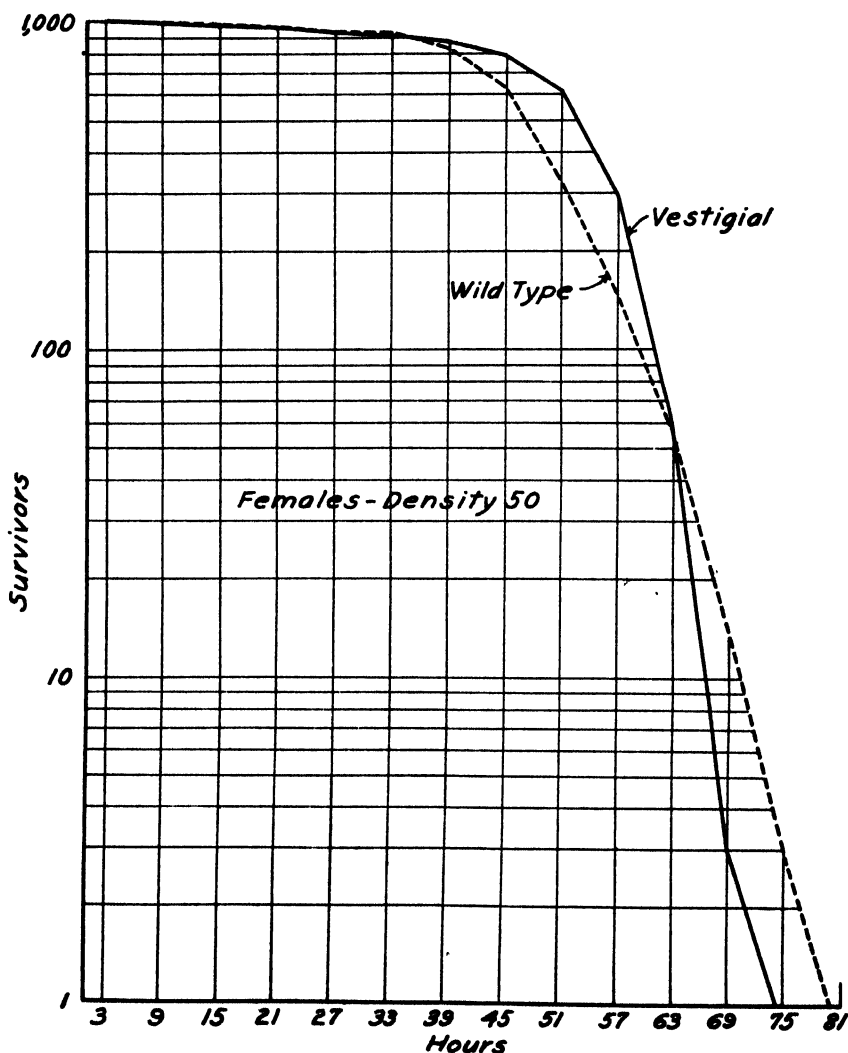


FIG. 2. Like Fig. 1, but for females.

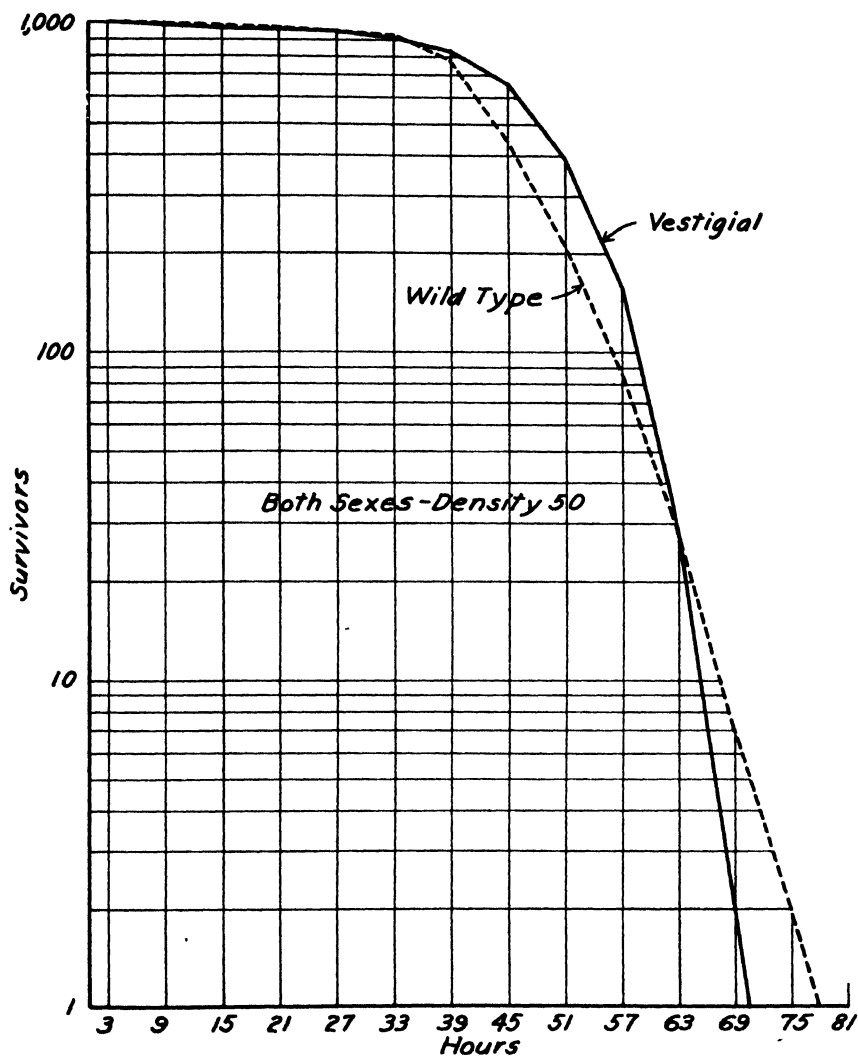


FIG. 3. Like Fig. 1, but for both sexes combined.

with what happens under normal conditions of feeding, as we have shown in a number of papers, and particularly with all the refinement of a life table in the study preceding this (66), the normal expectation of life at emergence is:

For wild type ♂♂, 45.8 days
 For wild type ♀♀, 48.0 days
 For vestigial ♂♂, 14.1 days
 For vestigial ♀♀, 19.8 days

In other words, the mean duration of imaginal life, or expectation of life at emergence, is approximately *three times* as great, under normal feeding conditions, in the wild type fly as it is in pure vestigials. Under complete starvation the two are nearly identical in absolute duration of life, and what slight advantage there is favors the vestigials. What we believe to be the significance of this result will be discussed farther on in the paper.

Variation in Duration of Life under Complete Starvation

An examination of the last columns of Tables III and IV shows that the coefficients of variation for duration of life under conditions of complete starvation are all *under 25 per cent.*, and a half of the 18 coefficients are under 20 per cent. These values indicate a *much lower relative variability in duration of life under starvation than under normal feeding*. This is demonstrated by comparing the coefficients of variation in Tables III and IV with any that we have published in earlier papers in this series. Take for example the coefficients of variation for duration of life in inbred lines as given in the second of these studies (32). There are given in that

TABLE V

COEFFICIENTS OF VARIATION IN DURATION OF LIFE IN (a) LINE BRED FLIES UNDER NORMAL FEEDING CONDITIONS, AND (b) FLIES UNDER COMPLETE STARVATION

Magnitude of coefficients of variation (in per cent.)	a. Under normal feeding	b. Under starvation
10-19	—	9
20-29	1	9
30-39	8	—
40-49	5	—
50-59	2	—
60-69	3	—
70-79	1	—
80-89	1	—
Totals	21	18

paper 21 coefficients of variation in duration of life. These are distributed in magnitude as shown in Table V. The distribution of the coefficients of Tables III and IV of this paper is inserted for comparison.

These two distributions are shown graphically in Fig. 4.

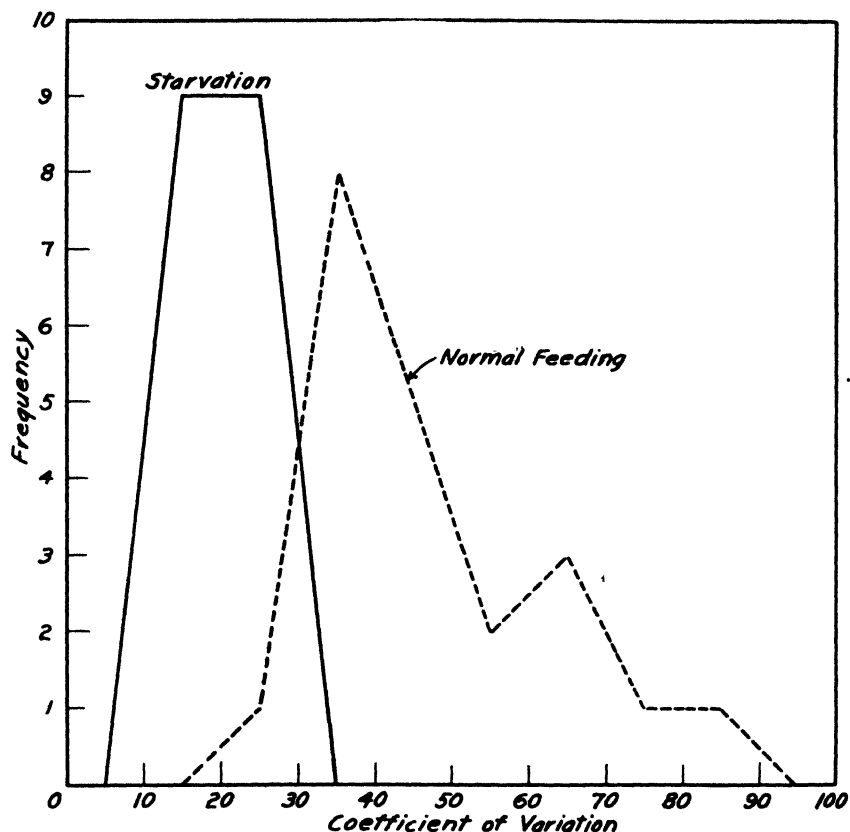


FIG. 4. Frequency polygons showing the magnitudes of the coefficients of variation in duration of life of *Drosophila* under starvation (solid line), and normal feeding (broken line).

The much reduced relative variability in duration of life under starvation is apparent. In fact it may be said that *roughly* one half of the variation observed among individual flies in the duration of life disappears when the varying environmental factor involved in and incident to the gaining of energy and renewal of body substance by feeding is annulled. Considering the extremely constant environmental conditions in all respects under

which these starvation experiments were carried out, it seems reasonable to conclude that at least the major part of the residual individual variation in duration of life observed therein (expressed by coefficients of variation of about 20 per cent.) must represent inborn individual differences in the organization of the mechanism which we call *Drosophila*, due allowance being made for the random fluctuations of a magnitude indicated by the probable errors.

Sex Differences in Duration of Life under Complete Starvation

From Table III we have the following systems of sex difference in the means.

		<i>Female mean—Male mean</i>	
<i>Wild Type.</i>	Density 5;	45.03 — 44.50 = + .53 ± .51	(1.0)
	Density 50;	46.94 — 42.21 = + 4.73 ± .54	(8.8)
	Density 100;	50.89 — 44.30 = + 6.59 ± .49	(13.5)
<i>Vestigial.</i>	Density 5;	48.72 — 43.97 = + 4.75 ± .56	(8.1)
	Density 50;	50.31 — 44.07 = + 6.24 ± .59	(10.6)
	Density 100;	52.47 — 44.74 = + 7.73 ± .46	(16.8)

It is evident that the females have a longer mean duration of life than do the males under conditions of complete starvation. The differences are all positive in sign, and five out of the six are eight or more times their probable errors. It seems safe to conclude that in general the sex differences are larger and steadier than could reasonably be supposed to have arisen from the fluctuations incident to random sampling alone. It is important to note that the direction of the sex differences (female longer-lived than male) is the same under starvation as under normal feeding. The significance of this result will be discussed more fully farther on.

Turning to the question of sex in relation to individual variation in duration of life we have to consider first following system of differences in the standard deviations of Table III.

Female standard deviation—Male standard deviation

<i>Wild type.</i>	Density 5; $8.91 - 8.77 = + .14 \pm .35$ (.4)
	Density 50; $11.08 - 7.69 = + 3.39 \pm .38$ (8.9)
	Density 100; $10.73 - 7.64 = + 3.09 \pm .35$ (8.8)
<i>Vestigial.</i>	Density 5; $11.31 - 8.24 = + 3.07 \pm .39$ (7.9)
	Density 50; $12.12 - 8.95 = + 3.17 \pm .41$ (7.7)
	Density 100; $9.98 - 7.30 = + 2.68 \pm .32$ (8.4)

In all cases the females exhibit a higher absolute variability in duration of life than do the males. In all except the first comparison the differences are more than seven times their probable errors. In wild type flies at density five the sex difference in standard deviation is insignificant.

For the coefficient of variation we have the following system of sex differences:

Female Coefficient of Variation—Male Coefficient of Variation

<i>Wild type.</i>	Density 5; $19.79 - 19.70 = + .09 \pm .83$ (0.1)
	Density 50; $23.61 - 18.23 = + 5.38 \pm .87$ (6.2)
	Density 100; $21.09 - 17.24 = + 3.85 \pm .75$ (5.1)
<i>Vestigial.</i>	Density 5; $23.22 - 18.74 = + 4.48 \pm .87$ (5.1)
	Density 50; $24.09 - 20.31 = + 3.78 \pm .90$ (4.2)
	Density 100; $19.03 - 16.31 = + 2.72 \pm .68$ (4.0)

It is evident that in relative as well as absolute variability in duration of life under complete starvation the female is more variable than the male. All the differences are positive in sign, and all except the first may be safely regarded as larger than would probably arise from random sampling alone.

The Influence of Density of Population upon Duration of Life under Complete Starvation

In planning the present series of experiments it seemed desirable in the light of our earlier results (74) to employ at least three densities, one of which should be below the optimum density for fed flies, one at about the optimum and one well above. With this in mind densities of 5, 50 and 100 flies per one ounce bottles were chosen. The question was whether there would be dif-

ferences in duration of life under these three densities comparable relatively to the marked ones that we have found in fed flies. From Table I of (74) we have for fed flies:

Mean duration of life at density	4	=	29.64 ± .60
Mean duration of life at density	55	=	40.66 ± .53
Mean duration of life at density	105	=	24.94 ± .32

Thus from the low density to the optimal there is an increase in mean duration of life of $11.02 \pm .80$ days, or 37.2 per cent. above that at the low density. From the optimum density to a fairly high density of 105 flies per bottle there is a decrease in mean duration of life amounting to $15.72 \pm .62$ days, or 38.7 per cent. below that at the optimum. These are large differences, and our certainty as to their reality in fed flies has been firmly established by extensive experiments, a complete account of which will shortly be published.

The following system of differences derived from Tables III and IV shows the influence of density under complete starvation:

Mean duration of life at density 50—Mean duration of life at density 5

<i>Wild type.</i>	♂ ♂	; 42.21 — 44.50 =	$-2.29 \pm .48$ hours	(4.8)
	♀ ♀	; 46.94 — 45.03 =	$+1.91 \pm .57$ hours	(3.4)
	Both sexes;	44.60 — 44.77 =	$-.17 \pm .38$ hours	(0.4)
<i>Vestigial.</i>	♂ ♂	; 44.07 — 43.97 =	$+.10 \pm .48$ hours	(0.2)
	♀ ♀	; 50.31 — 48.72 =	$+1.59 \pm .65$ hours	(2.4)
	Both sexes;	47.20 — 46.36 =	$+.84 \pm .42$ hours	(2.0)

Mean duration of life at density 50—Mean duration of life at density 100

<i>Wild type.</i>	♂ ♂	; 42.21 — 44.30 =	$-2.09 \pm .42$ hours	(5.0)
	♀ ♀	; 46.94 — 50.89 =	$-3.95 \pm .59$ hours	(6.7)
	Both sexes;	44.60 — 47.69 =	$-3.09 \pm .38$ hours	(8.1)
<i>Vestigial.</i>	♂ ♂	; 44.07 — 44.74 =	$-0.67 \pm .44$ hours	(1.5)
	♀ ♀	; 50.31 — 52.47 =	$-2.16 \pm .60$ hours	(3.6)
	Both sexes;	47.20 — 48.51 =	$-1.31 \pm .39$ hours	(3.4)

It is at once evident that density *per se* produces no such effect upon duration of life under complete starvation as it does under conditions of normal feeding. As between densities 5 and 50 the differences in the case of

the wild type flies are irregular as to sign, and near the border line as to significance. Taking both sexes together the difference is plainly not significant. Yet approximately the same difference in density has associated with it, in the case of fed flies, a difference in mean duration of life of about 37 per cent.

In the case of the vestigials the density difference from 5 to 50 has associated with it a *slightly* higher mean duration of life in the latter density, but the differences are too small to be statistically significant. The tabled differences might easily have arisen from chance fluctuations in sampling alone.

Turning to the density difference between 50 and 100 it is seen that the sign of the duration of life differences is in all cases negative. In other words, under conditions of complete starvation both wild type and vestigial flies have a mean duration of life of four to five per cent. longer at density 100 than at density 50. This is directly opposite to what is found in fed flies, as has already been pointed out. The differences are also *relatively* smaller. But in the case of the wild type flies they are absolutely large enough to be probably significant statistically. In the case of the vestigials, the differences are less probably significant.

Altogether it is apparent from these results that a considerable part of the differences in duration of life associated with differences in density of population in the case of fed flies *must be directly connected with feeding*, because they become very much smaller relatively under conditions of complete starvation. There seems no escape from this conclusion. It is important in two ways. In the first place it suggests at least a partial explanation for some of the puzzling results which we have demonstrated in regard to the effect of density upon duration of life; and in the second place it points the way to new experimental points of attack upon the density problem.

There is a slight, but still fairly definite suggestion of a tendency for duration of life under conditions of com-

plete starvation to *increase* as density increases, up to a density of 100 flies per one ounce bottle at least. This is especially marked in the case of vestigials. The determination of what this means relative to the general problem of density effects must await further experimentation, but the probable fact is worthy of some emphasis now, lest it be overlooked in future discussions of the density problem.

There appears to be very little effect of density of population upon *variation* in duration of life, under conditions of complete starvation.

The Form of the Life Curve under Starvation

We come now to the consideration of the chief problem set at the outstart of this investigation. Do wild type and vestigial flies show the same form of life curve under conditions of starvation and of feeding? To get light on this point it is necessary to put the two cases on a basis of centile age instead of absolute age, according to the plan described in earlier studies in this series (*cf.* 61, 63 and 66). This is done in Figs. 5 and 6. In Fig. 5 the centile age survivorship curve for Line 107 wild type males under conditions of normal feeding (66, Table VI, p. 79) is compared with the centile age survivorship curve for wild type males of the same line under starvation conditions and initial density 50. In Fig. 6 the centile age survivorship curve for vestigial males under normal feeding (66, Table VI) is compared with the corresponding curve for vestigial males under starvation conditions and initial density 100. It is not thought necessary to compare the curve for females or for other densities, because nothing different in principle would be brought out. Also it has not been deemed necessary for present purposes to graduate the starvation curves. They are sufficiently smooth as they are to show clearly the essential point.

An examination of these diagrams makes it clear that so far as wild type flies are concerned the form of the

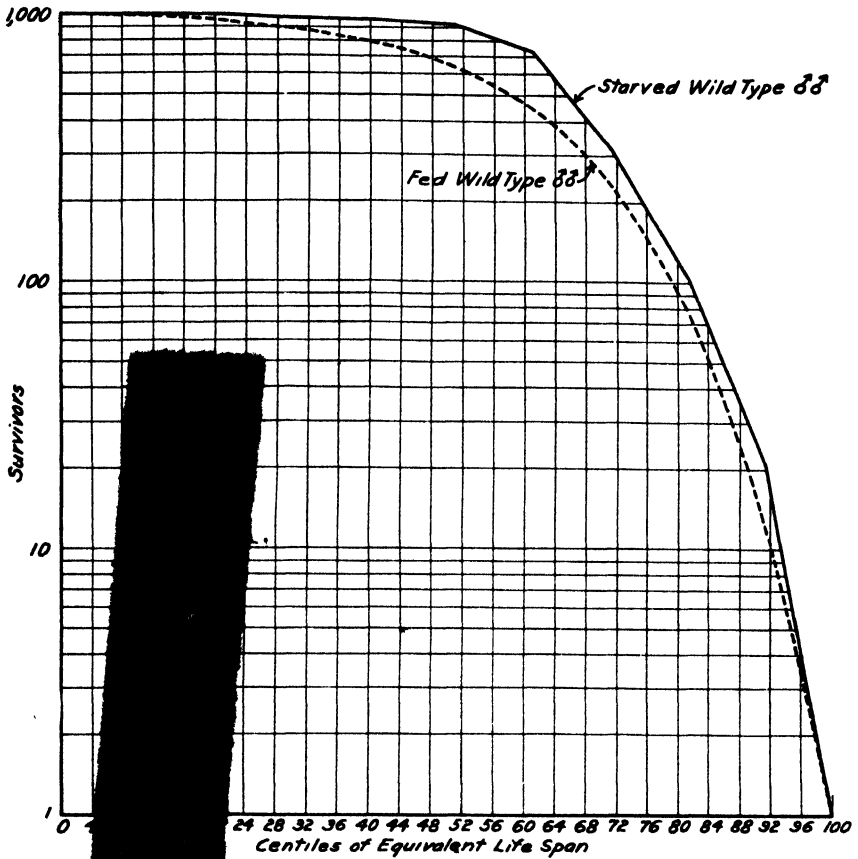


FIG. 5. — (a) the survivorship curves on a centile age base of (a) starved wild type ♂♂ (solid line) and (b) fed wild type ♂♂ (broken line).

life of the flies is substantially the same whether the individuals are fed or starved. The specific death rates are somewhat higher during the first half of the equivalent life span in the case of the starved flies, but at equivalent ages after about centile 60 the rates are practically identical. In the earlier half of the span the differences are, however, insignificantly small, in all probability.

This is very different with the vestigial flies (Fig. 6). The starvation curve is, as has already been seen, very similar in shape to the wild type starvation curve, which in turn has the normal form, common to *Drosophila* and other forms under normal feeding conditions. But this is quite different from the form

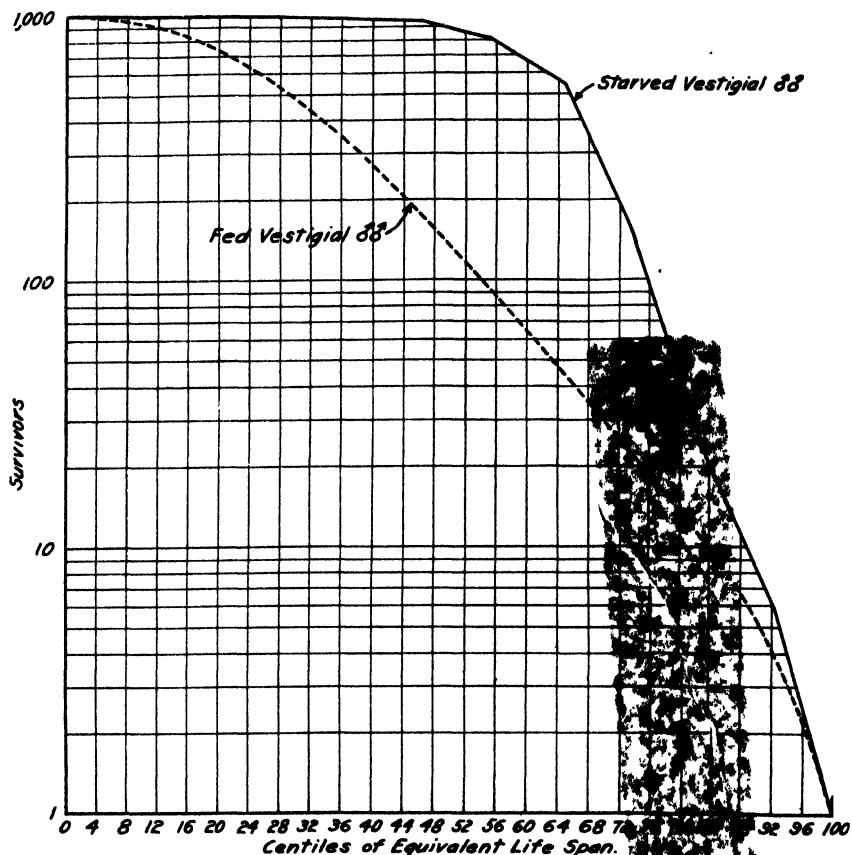


FIG. 6. Like Fig. 5, but for vestigial ♂♂.

of the survivorship curve upon a centile axis for vestigials under conditions of normal feeding. The latter curve, as we have already pointed out in (65), approaches closely to the straight diagonal on an arithmetic scale which would be given by absolutely uniform specific death rates throughout life.

So then it appears that while it would be possible to make a very close approximation to the normal life table of normally fed flies of *wild type* by observing only the mortality of wild-type flies under complete starvation, it would be wholly impossible to do this for *vestigial* flies. This suggests that what we call normal laboratory feeding conditions do not permit vestigial flies to reach their full innate potentialities in respect of duration of life. It

also suggests that just as vestigials live quite as long as wild-type flies under complete starvation, it might be possible to devise a system of *feeding* vestigials which would enable them to live just as long as fed wild-type flies. Preliminary experiments indicate clearly that this is the fact.

The genetic significance of the facts brought out in this section will be discussed in the next section.

Discussion

From the data given by Lutz (13) regarding the duration of life of *Drosophila* (wild type) given water, but no food, we have calculated the following constants for comparison with those of this paper:

Mean duration of life = (♂ ♂) $66.31 \pm .60$ hours; (♀ ♀) $68.61 \pm .62$ hours
 Standard deviation = (♂ ♂) $9.51 \pm .42$ hours; (♀ ♀) $10.17 \pm .44$ hours
 Coefficient of variation = (♂ ♂) $14.35 \pm .65$ (♀ ♀) $14.83 \pm .65$

Comparing these values with those of Table III, *supra*, it is evident that the addition of drinking water has considerably increased the mean duration of life under complete absence of food. This result is probably due to two things. First, animals generally endure starvation better if they have water to drink than if they lack it, even though the atmosphere in the latter case is saturated with moisture, so that no desiccation occurs. Second, it is probable that the water furnished contained minute amounts of material in solution, and bacteria and yeasts washed off the bodies of the flies themselves which served in some measure as food.

Lutz's experience confirms ours in respect of the reduced *variation* in duration of life under conditions of starvation. The standard deviations are of about the same area of magnitude as ours, while, of course, the coefficients of variation are lower, owing to the larger means.

The most striking general result of the present study is the finding that while under normal feeding conditions

wild-type flies live on the average about three times as long as vestigials, nevertheless when placed under conditions of complete starvation the vestigials live as long as the wild type. This fact is of great importance from the point of view of genetics. It is a specific example of the general principle that the somatic expression of any genetic factor in any particular case is in part a function of the general environmental level which prevails in that case. It has been demonstrated (57, 62) that *under the standard feeding conditions for laboratory-bred Drosophila* the gene for vestigial has as a part of its somatic expression a very considerably reduced duration of life as compared with the wild type. There are few cleaner cut cases of Mendelian segregation to be found in the whole literature of genetics than that upon which the above statement is made. Yet the present study shows that the whole of that part of the somatic expression of the vestigial gene which is differential in respect of duration of life disappears under another system of "feeding" wild-type and vestigial flies (namely, complete starvation). This fact does not in the least invalidate the earlier results cited on the inheritance of duration of life. Those results are *facts* just as much as the present ones. It merely emphasizes once more, in a rather striking way, the extraordinary caution which is always necessary in interpreting the results of genetic experiments. It also, of course, points the way to new and very promising lines of further experimental attack upon the problem of duration of life.

The general genetic principle enunciated above has been recognized, formally at least, by many workers. A case which in some important respects of principle closely parallels the one here presented has lately been described by Metz (77), having to do with the effect of temperature on the expression of the mutant gene "bent," in *Drosophila*.

Pursuing this same subject of the influence of environmental conditions upon the expression of inborn, genetic

characteristics a little further, it is of interest to note that the same kind of difference in mean duration of life which is observed between the two sexes under conditions of normal feeding is also found under starvation. We interpret this result to mean that the innate constitutional differences which distinguish the two sexes are of a much more fundamental character than those consequent upon the presence or absence of the mutant gene vestigial. Those differences in innate constitutional make-up or organization which make a female *Drosophila* live longer on the average than a male are too deep-seated to be upset by the most extreme differences in feeding (full feeding versus complete starvation).

Summary

In this paper are given the results of the determination of duration of life in each of 3,632 individuals of *Drosophila*, under conditions of complete starvation. The chief results are:

1. Under complete starvation the mean duration of life and the form of the life curve are practically the same in vestigial as in normal wild-type flies, though under conditions of full feeding the wild-type flies live roughly three times as long as the vestigials.

2. There is a much reduced relative variability in duration of life under conditions of complete starvation than in conditions of full feeding.

3. The normal relation between the sexes in respect of mean duration of life (females longer-lived than males) observed under full feeding, is preserved under conditions of complete starvation.

4. Females are more variable than males, both absolutely and relatively, in duration of life under complete starvation.

5. The relation of duration of life to density of population is quite different under complete starvation from what it is under full feeding.

The genetic significance of these results is discussed.

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THE PHYSICO-CHEMICAL CONDITIONS OF MORPHOGENESIS*

RALPH S. LILLIE

THE attainment of definite or final form in animals and plants (either of the whole organism or of its special parts) usually comes at the end of a more or less prolonged sequence of growth processes, accompanied by a progressive and orderly deposition of permanent structure. This emergence of definite structure in the growing mass, with associated definite function, is the process usually called "differentiation." The essential problem of morphogenesis is the problem of why the form and structure thus attained should be constant for a given species, *i.e.*, should recur constantly in successive generations. This problem, as thus defined, becomes almost indistinguishable from the problem of heredity, the chief data of which are structural characters, or physiological characters based on structural characters. The physiological study of this problem is still in an early stage. We know that the adult structural characters are correlated in a definite manner with the structural characters of the germ cells. In particular, the relation between the number, structure and behavior of the chromosomes and the later appearance of somatic characters has been investigated lately in great detail and with a striking degree of success. We can now predict with a fair degree of statistical accuracy what special structural characters will emerge at the end of the morphogenetic sequence when two germ-cells of a given Mendelian constitution unite in fertilization. Such possibility of prediction shows that the series of formative processes is dependent in its finer details upon the nature of the initial combination occurring in maturation and fertilization. In itself, how-

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ever, it tells us little concerning the detailed physiological nature of these processes. The latter problem is one for physiology.

Briefly the physiological problem may be defined as the problem of the nature of the physico-chemical processes concerned in the formation of organic structure. In our study of this problem we omit all consideration of final causes and assume that at every stage the efficient causes are physical. The constancies observed in external nature are physical constancies and have their root in physical conditions. It is clear that the existence and perpetuation of organisms depend upon processes whose essential features are constancy, determinability and order. Stability of process is the primary requisite for constancy of outcome in any kind of development. Morphogenetic processes exhibit the highest form of order. As Santayana puts it, "Only order can beget a world or evoke a sensation."

That natural processes under defined conditions pursue their course with exactitude is simply a fact of observation. This, which we recognize as true of simple physical sequences, is equally true of the complex processes of growth and morphogenesis which begin with fertilization and end with the emergence of the imago from the cocoon. Such sequences resemble simple physical sequences in the one fundamental respect, namely, their determinability. We know of many simple and regular morphogenetic sequences in inorganic nature, for example, the sequence by which a supersaturated solution (not inaptly called a "mother liquor") yields a crop of crystals of definite form when sown with solid fragments of the dissolved compound. Here we are bringing into comparison (or at least into simultaneous consideration) processes which have in common but one feature, namely, the production of a physical complex, having a definite structure and form, as the end-result of an accumulation or accretion which starts from a minute germ or center structurally much simpler than the final product. Both instances ex-

emphify what is called "germ-action," a term transferred to physical science from biological observation. It is especially to be noted that the nature of the formative process is determined both by the physico-chemical constitution of the original center and by the conditions in the surroundings; *i.e.*, two sets of factors, internal (those initially present in the germ) and external, are concerned. Under the same conditions in the surroundings two living germs or two crystals may carry through entirely different sequences of morphogenetic processes. A solution supersaturated with more than one compound separates out crystals of the compound which is introduced in solid form; similarly, a yeast or mould introduced into a culture medium forms a center of construction of organized structure of a specific kind. Both crystal and organism are accretions, their materials are collected from the surroundings; both attain definite form, structure and physical properties at the end of a sequence, more or less complex, of form-determining processes. This fundamental resemblance can not fail to be significant, however great may be the differences in the details of the two sequences.

It will be understood that in thus reverting to the time-honored comparison of inorganic crystallization with organic morphogenesis, I am not implying any complete identity of the two processes. What I do imply, however, is that the conditions which render possible definite form-determination in the inorganic system are also an indispensable part of the conditions underlying the specific form-determination in the living system. Without constancy in the finer elements of organic structure, constancy in the structure of the whole organism would hardly be possible. Underlying the constancy of organic form we must assume the constancy of sub-organic form. This means constancy of the antecedent form-determining conditions. The synthesis by which the adult organic form is constructed with such regularity is based upon the synthesis of those specific chemical compounds which

compose its finer structural elements. These compounds as they separate in solid form to form the structural substratum of protoplasm adopt a finer structure which we have every reason to believe is in the last analysis crystalline. It is known that in many cases the separation of proteins (the chief structure-forming compounds of protoplasm) from solution is in crystalline form *e.g.*, fibrinogen solutions deposit crystals of fibrin when they clot; the internal structure of many gels has been shown to be crystalline; and crystals are formed in the "salting-out" of many proteins. In general, underlying and determining crystalline growth is a process of surface deposition which is in the nature of an adsorption; in adsorption the adsorbed molecules adopt regular orientation at the surfaces, as the recent work of Langmuir, Harkins and Adam has shown. The regularity of the crystal-form is determined by this regularity of surface orientation. All the indications are that organic growth also proceeds by the deposition of new material at the surfaces of structures already laid down. This process implies the formation of new structure; and the special nature of this structure is determined by the chemical composition of the material which is thus deposited.

Crystalline structure is the visible evidence of the orderly arrangement of the atoms in molecules and of the molecules in molar masses. We know from the results of the X-ray analysis of crystal structure that the atoms in solids have fixed positions relatively to one another, and that these positions depend on chemical constitution. The whole structure formed by the cohering molecules has the regularity of an accurately ruled grating; this structural regularity is what makes possible this mode of physical analysis. The atomic groups which, when detached from one another (*e.g.*, in solution), we call molecules, have in crystals a close-packed arrangement with the axes parallel and the interatomic distances and angles constant and measurable. The definite form adopted by the molar structure—the crystal—is the expression of

this regularity in the structure of the individual molecules and in their manner of coherence. The existence of cleavage planes, as well as of other constant properties, such as tenacity, elasticity, refractive index, etc., is most readily understood on this conception. Cleavage planes, for example, are the planes along which the adjacent layers of molecules are most readily separated from one another. In fusion or solution the molecules lose regular orientation as they lose coherence; the form characters of the aggregate are then lost. The original form is regained when the range of molecular movement is reduced by lowering the temperature, or when the solvent is removed. The morphogenetic process by which the crystal is formed is thus characteristically reversible; the same is true of certain types of organic morphogenesis. Recently the crystalline structure of relatively complex organic compounds like naphthalene has been investigated with success by Sir William Bragg, and the results are in close conformity with the conception of molecular structure derived from a consideration of purely chemical properties. Astbury's recent studies of the X-ray structure of tartaric acid bear out completely Pasteur's conception of the asymmetric arrangement of the atoms; *i.e.*, there is direct optical evidence of the existence of two atomic arrangements, one the mirror-image of the other.¹ Two conclusions follow, both applicable to our biological problem, first, that the finest elements in a solid structure have a regularity of a definite repetitive kind, and second, that in complex organic compounds the special structure adopted is determined by the stereochemical constitution of the molecules.

The arrangement of the molecules in solid structures is determined by the specific chemical constitution of the molecules, and this is determined by the dimensions, valences, force-fields and other characteristics of the combined atoms. The characteristics of each atom, accord-

¹ Cf. W. T. Astbury: "Analysis of crystal structure by X-rays." *Science Progress*, 1923, Vol. 17, p. 386.

ing to the electron theory, are determined by the relative numbers, arrangements and motions of the electrons grouped about the central positive nucleus. From crystalline or molar structure to molecular structure, and from molecular to atomic structure, and from atomic structure to the properties and motions of the electrons, these characteristic relations of interdependence extend. These relations are asymmetrical in the logical sense, *i.e.*, the characteristics of each larger product of synthesis are based upon or presuppose those of the smaller; but the smaller exist and have their properties independently of the larger. So that while in analysis we have a convergence to simplicity (to use Whitehead's phrase²), in synthesis unlimited possibilities of diversity present themselves. Although many of these possibilities have been realized in nature, no limit can be assigned to the synthetic process, and evolution is still in active progress.

Professor Niels Bohr is reported to have said in his recent Yale lectures that a true interpretation of atomic structure opens the way to a reversal of the usual order of scientific explanation. Our study of the universe may begin with the smallest particles of matter and work upward, *i.e.*, synthetically, instead of downward (as hitherto) from large masses to the minute. Proceeding in such a manner we might retrace in outline the process of creative evolution by which the present status of the physical universe has been reached. We should see how the synthesis of molecules from atoms has followed the synthesis of atoms from protons and electrons. The synthesis of molar or solid structure (which is essentially crystalline structure) has followed as the next step in synthesis. Finally, we should come to the synthesis of special interest to biologists, that by which organic structure with its correlative physiological activity has arisen from a combination of inorganic materials and energies.

The subject of our present discussion is morphogenesis in the biological field. Morphogenesis, the production of

² "The Concept of Nature."

definite form and structure, is a constant expression or result of vital activity, characteristic for each species. The materials that form the permanent structure of any organism are either synthesized by the organism or collected from the surroundings; in either case they are deposited in solid form in definite situations during the period of growth. The order and locus of their deposition are determined by uniformly acting factors, partly physical, partly physiological. Summarizing the foregoing brief analysis, we may say that the fundamental or primary morphogenetic factors are of a general or non-specific physical kind, of the same nature as those underlying crystallization, and that superposed on these simpler physical factors are more complex secondary or "physiological" factors which are specific and vary from organism to organism.

Let us now briefly consider the nature of the more specifically vital or physiological factors of morphogenesis. The chief questions are the following: (1) What is the special physical and chemical nature of the material which forms the living or organized structures? and (2) why does it assume the form which we observe in each special case? These problems may be regarded as the special problems of morphogenesis as distinguished from the other general problems of physiology from which, however, they can not be separated. In a broad sense these problems are the same in all organisms, although in higher organisms the simple fundamental conditions are likely to be obscured by special complications which from the physical point of view are mainly incidental. The living material is plastic and may adopt almost any form consistent with its fundamental requirements, access of nutrients and oxygen and removal of waste metabolic products.

In seeking factors common to all cases of morphogenesis we may therefore find it more instructive to consider first the simpler organisms or single cells. Why do single cells under favorable conditions of nutrition and

oxygen-supply attain definite form and dimensions? In higher organisms cellular form and dimensions are constant within certain well-defined limits of variation. The nuclear-plasma relations are also constant; the same is true of the structure and mode of deposition of the finer structural elements, such as the fibrils in muscle cells. Bacteria, yeast cells and protozoa growing in culture media also attain constant form, structure and dimensions, resembling in this respect the cells of higher organisms. Such general relations are usually interpreted as indicating that when in growth the ratio of surface to volume is reduced below a certain volume, division ensues. The surface-volume ratio thus passes through a rhythmical variation about a certain mean, decreasing at the interdivisional periods and increasing at division. Under normal conditions, this mean is maintained with constancy, and this constancy ensures the constancy of the cellular dimensions. But evidently underlying this regulative process are the metabolic reactions of the protoplasm, which determine the special nature of the added material. These must have a similar cyclical character.

Robertson's recent researches³ render it probable that in protozoa growth is controlled by the production within the nucleus of a substance which has an autocatalytic relation to certain metabolic reactions essential to growth. The distribution of this substance between nucleus and cytoplasm and between cytoplasm and external medium occurs periodically at times of division. This is the time at which nuclear and plasma membranes undergo increase of permeability. The observed sequence, growth to a certain size, division and resumption of growth to the same size, may thus in a measure be understood, division corresponding to a stage when a certain accumulation of constituents has occurred within the cell. The concentration-relations of such a substance will be deter-

³ Cf. Robertson: "The Chemical Basis of Growth and Senescence," Philadelphia, 1923.

mined both by the nuclear-plasma volume-ratio and by the ratio of the general cytoplasmic surface to the volume enclosed (or specific surface of the cell); in this way, a size-regulating mechanism has been evolved, depending ultimately on conditions of chemical equilibrium.

Regulation of cell-size is, however, only one elementary feature of morphogenetic processes. The regulation of cell-structure is of more immediate interest. We have to account for the constant form-characters of the structures which are laid down within the cell as it grows and develops. Now, broadly speaking, when we consider the relations, already briefly sketched, between molecular structure and the molar structure of materials in the solid state, we can see that the solid metabolic products appearing in the cell must, as they are deposited, tend to form structure of a constant kind. The reason why these structures are specific and constant for each cell must, therefore, be intimately related to the fact that their chemical composition is also specific and constant.

The relation of proteins to specificity has often been discussed. There is a correspondence between the diversity of native proteins and the diversity of native organic species, although proteins are more numerous than species, since most organisms have a variety of specific proteins. When corresponding proteins from two species of animals are chemically similar (as shown by precipitin or anaphylaxis tests), we find also that the species are structurally and physiologically similar. Proteins from nearly related animals, when separated from solution under conditions favorable to crystallization, form aggregates which are structurally similar; this has been shown most clearly by Reichert and Brown in the case of hemoglobins. What is true for these proteins must be true for others. Similarly constituted compounds form similar structural aggregates. If this is the case *in vitro* we may assume that it is also the case when the compounds are laid down within or between the cells to form organic structure. Chemical similarity determines struc-

tural similarity, and this secondarily determines physiological similarity. It is true that many other compounds besides proteins are present in protoplasm, and that the nature and proportions and concentrations of these are also constant for a particular species of cell; these compounds also play a part in determining structure. Yet the peculiar physical properties of proteins seem to determine what is specific in protoplasmic structure, *i.e.*, why one species of cell differs characteristically from another. For example, that the plasma membrane of one species of blood corpuscle is structurally different from that of another is shown by the differences in physical properties (iso-electric points, permeability, resistance to hemolysis, etc.); and that there is a corresponding difference in the structural proteins is shown by the existence of specific hemolysins.

How are the proteins formed within the cell? And what determines the peculiar specificity which they show? Why should one species of cell form constantly proteins of a definite stereochemical configuration? These problems are not yet solved. We merely know that from the same mixture of amino-acids one cell synthesizes one set of proteins, and another cell another set. In this process we have again a form of germ action. Something analogous to accretion occurs; external compounds are attracted and enter the cell; but this accretion is associated with a characteristic sorting activity, combined with dehydrolytic condensation, as a result of which the amino-acids are linked in definite order. Typically, this order corresponds to that characteristic of the structural proteins already present. For example, we observe that the fibrils of an exercised muscle cell increase in size and number. This process is not identical with crystallization, but it has its affinities with this process, *i.e.*, underlying it are processes of the same nature as those determining crystallization. The amino-acids are not only attracted in some manner to the structure already present, but they are dehydrolytically condensed to form the

same structural proteins. Of the chemical details of this process we are still ignorant. Apparently oxidations are necessary, especially of carbohydrate. We know that a separation and rearrangement of amino-acids occurs within the cell in many cases, *e.g.*, those in which protozoa or bacteria grow in a medium containing protein but no free amino-acids. The cells, however, contain proteases, so that conditions for hydrolysis are present. What is not yet understood are the conditions determining the specific rearrangement.

Yet these conditions must be understood if we are to understand growth and morphogenesis in any fundamental sense. Some light is thrown on the problem by the facts of immunology. We know that the introduction of foreign proteins into the circulation of a higher animal results in the formation, by the cells of the animal, of compounds (antibodies) which are similar or complementary in their configuration to those introduced. This seems to indicate that in the presence of one protein the formation of another of similar structure is promoted or catalyzed. It is true that anti-bodies are not identical with antigens; they are, however, structurally closely related, so that close adhesion and union occur when both are present. The essential fact is that there is a correspondence in configuration between the introduced compound and the compound which is synthesized by the living protoplasm under its influence. Similarly, in the growing muscle cell the proteins already present in some manner enable metabolism to produce identical protein and hence identical structure. The precise physico-chemical conditions of this reduplication are unknown. We may leave the problem in this stage, the stage of definition. Its solution can only follow further study and experiment.

Two years ago at a meeting of this society Professor Muller called attention to the bearing of the d'Herelle phenomenon on a related problem, the problem of the multiplication of genes.⁴ In this phenomenon, first ob-

⁴ H. J. Muller: AMER. NATURALIST, 1922, Vol. 56, p. 32.

served by d'Herelle in the bacillus of dysentery, the characteristic feature is the continued reproduction of a substance, specifically lethal to the organism, in response to the introduction of a small quantity of the same substance into the culture medium. Similar phenomena are known in other organisms, for example, the multiplication of contagious viruses in certain plant diseases, *e.g.*, mosaic disease of tobacco or infectious chlorosis of Malvaceae;⁵ the growing parts produce the specific toxic compound, which is identified by its lethal action on other growing parts. The synthesis of a new specific compound, ordinarily not present in the protoplasm, is promoted or autocatalyzed by its own presence. These lethal compounds, as well as the bacteriophage in the d'Herelle phenomenon, multiply only during the process of cell-multiplication, *i.e.*, in association with the other specific syntheses of the cell-protoplasm. The lethal character is incidental, like the lethal character of certain genes; if the compounds were not lethal they would not be detected and their specific nature established. What is of interest is that their presence forms the condition for the metabolic synthesis of more material of the same kind. This property is probably universal in protoplasm, and apparently constitutes the basis of its characteristic self-multiplying property or power of growth.

Finally, we come to a consideration of the conditions determining the special and often complex form of multicellular organisms. Here the factors are correspondingly complex and our knowledge is in an early stage. It is clear, however, as d'Arcy Thompson has recently pointed out,⁶ that the form adopted by any growing animal or plant is an index of the relative rates of growth in different regions. The problem resolves itself into the problem of the conditions determining the local differences in rate of growth. Only when we understand the nature of

⁵ Cf. the recent review by C. E. Simon, "The filterable 'viruses,'" *Physiol. Reviews*, 1923, Vol. 3, p. 483, for further details and references.

⁶ "Growth and Form."

growth and the factors determining its rate shall we be in a position to understand why each organism assumes a definite form as it develops. Certain generalizations drawn from the observation of morphogenetic processes have a fundamental significance in relation to this problem; for example, the law of anteroposterior development, which expresses a generally observed tendency for the anterior regions of bilateral animals to grow and differentiate more rapidly than the posterior regions. Child has shown that this gradient in the rate of growth is correlated with a gradient of susceptibility to poisons and with a gradient of oxygen consumption and of carbon dioxide production. There is also a gradient of electrical potential. The secondary axes of the growing organism appear to have similar properties. In general, the presence of growing apices or growing zones is a highly characteristic feature of multicellular organisms. The actively growing regions are definitely localized with reference to the chief axes; that these regions correspond with actively metabolizing regions is clear, apart from the evidence cited above, since growth is an expression of constructive metabolism. An additional fact of general significance is that such regions have typically a characteristic physiological influence on adjoining regions, repressing or inhibiting the growth of the latter. The whole structure thus exhibits a physiological polarity of a special kind; morphogenetic polarity is one expression of this polarity, the general conditions of which remain to be considered.

The physico-chemical conditions that determine this physiological or metabolic polarity are evidently of critical importance in the morphogenetic process. A combination of structural and active (*i.e.*, physiological) factors must be assumed. The first question to decide is whether this polarity is fixed by internal organization, or dependent wholly or in part on external conditions. Many attempts have been made to alter or reverse polarity by experimental means. Apparently in higher animals the

internal or constitutional factors are usually too fixed to admit of such change. In lower organisms, however, experimental reversal of polarity has been accomplished in a number of instances. These cases appear to be the most significant from the point of view of the physiology of morphogenesis. We know that the direction of growth, especially in plants, is readily influenced by external agencies, chemical, mechanical and electrical. In cut hydroid stems, differences of oxygen supply may determine morphogenetic polarity, as when the free end develops the hydranth, the buried end the stolon. Recently Lund has shown that the polarity of these stems may be controlled by the electric current;⁷ other recent evidence from both animals and plants has also illustrated the directive influence of the electric current on growth. And since organic polarity indicates metabolic or chemical polarity, we are led to consider the general conditions under which chemical polarity is exhibited in other natural systems.

Broadly speaking, the most general conditions determining chemical polarity—by which term we mean reciprocity and mutual interdependence of the chemical reactions in two regions of a reaction-system—are electrical. The difference between the chemical processes at the anode and at the cathode of a battery or electrolytic cell appears to present the closest inorganic parallel to the difference between the chemical processes at the growing and the quiescent regions of an organic structure showing polarity of growth. In the regenerating hydroid stem the hydranth-forming region is electrically negative to other regions, as Mathews and Lund have shown; and a similar polarity has been shown to exist in other cases. This fact is to be correlated with the fact that morphogenetic polarity may be reversed in certain cases by the use of electric currents of appropriate intensity.

The influence of electric currents on growth processes is one manifestation of the general electrical sensitivity

⁷ Lund: *Journ. Exper. Zool.*, 1921, Vol. 34, p. 471.

of living matter. This sensitivity is based on its partitioned or film-pervaded structure, *i.e.*, on the presence of polarizable (*i.e.*, semi-permeable) partitions or membranes surrounding and penetrating the living protoplasm. This condition is a feature of its emulsion-like or alveolar structure. Changes in the electrical polarization of these partitions occasion chemical reactions when an electric current passes through such a system. In a general sense it is not remarkable that electric currents should influence growth, since they influence so many other chemical processes in living protoplasm; this is best shown by the universal fact of electrical stimulation. Electrical stimulation has long been known to be a polar phenomenon, and the same is true for the directive influence of the electric current on growth.

The direction of the bioelectric currents ("growth currents") in growing organisms has a definite relation to the regions of rapid growth. Most observers agree that the rapidly growing regions are electrically negative relatively to slowly growing regions, *i.e.*, are the regions where the current (positive stream) of the bioelectric circuit enters the protoplasm from the surroundings. Now, in general, in any arrangement of two conductors separated by a polarizable surface and traversed by a current (*e.g.*, a metal-electrolyte combination) the region where the positive stream enters the chemically reactive phase is the seat of chemical reactions of an oxidative kind; this is illustrated in the oxidations occurring at the anode of a battery or electrolytic cell. If the cell-surface is comparable with an electrode surface, we should expect that the region where the positive stream enters the protoplasm from the surroundings would similarly be the region where oxidations are promoted. It seems probable that this is the general physico-chemical explanation of the association of electrical negativity with regions of active growth. The "negative" regions are those where the positive stream of the bioelectric circuit enters the protoplasm. Such regions are also the regions of most

active oxygen consumption. In Lund's experiments on the reversal of polarity in *Obelia* by the constant current, he found that the formation of hydranths from the cut hydroid stems was promoted at the ends facing the anode, *i.e.*, where the positive stream of the external current enters the protoplasm from the surroundings. Since in regeneration under the normal conditions the region of hydranth-formation is the region where the normal bioelectric current has this direction, it seems clear that the reversal of morphogenetic polarity corresponds to a reversal of the direction in which electric currents traverse the living system.

If the electric current promotes oxidations where it enters the living system from the surroundings, it should also promote processes dependent on oxidations, including synthesis and growth. The relation of oxidations to protoplasmic syntheses is not understood in detail. Some of the general physico-chemical conditions of intracellular oxidations appear, however, to be now well established. We know from the facts of electrical stimulation and narcosis and from the general dependence of metabolic reactions on protoplasmic structure that the chemical activity of living matter is a function of its polyphasic composition, *i.e.*, of the presence of phases or structural elements bounded by electrically polarizable surfaces or partitions. There are many indications that the syntheses underlying growth-processes occur at the surfaces of the protoplasmic phases; this is, in fact, implied by the susceptibility of these processes to electrical control. Nernst and others have shown that the electric current affects living protoplasm through its polarizing action, a fact indicating that the electrically induced reactions occur at the surfaces where polarization is altered by the current. The same is true of the reactions at metallic electrodes, which are also surface reactions. The narcotizability of growth processes by surface-active compounds is further evidence of this condition, since this form of narcosis appears to be dependent on the dis-

placement of oxidizable compounds from the protoplasmic surfaces, as indicated especially by Warburg's recent work.⁸

Warburg has shown that amino-acids undergo rapid catalytic oxidation at the surface of animal charcoal, although they are highly stable compounds in homogeneous solution. This fact is highly important from the present point of view, since it shows that the reactivity of these compounds, when adsorbed, *i.e.*, when under the influence of electrically polarized surfaces, is very different from their reactivity in homogeneous solution. If these compounds are readily oxidized under these conditions, it may be assumed that the readiness with which they enter into other reactions is also increased. It is true that the formation of proteins from amino acids is not an oxidative but a dehydrolytic process, but it appears to be dependent in some manner upon the intracellular oxidations. Just why oxidations, especially of carbohydrate, should be so essential to the dehydrolytic condensations underlying growth is not clear; a suggestive fact is that the action of dehydrating agents (hypertonic sea-water) in artificial parthenogenesis is similarly dependent on oxidations. Possibly the protoplasmic dehydrations depend on alternate oxidations and reductions; but this again is a problem which awaits solution. Specific synthesis requires a specific rearrangement of amino-acids; presumably the energy of the work thus performed comes from oxidation, but many features of the problem remain obscure.

The hypothesis that synthesis and deposition at the protoplasmic surfaces is the essential underlying condition of organic growth processes explains in a general way the relation of the electric current to growth; it also explains the interferences which result when highly adsorbable indifferent compounds (narcotics) are introduced into the protoplasmic system. The detailed nature of the formative process depends on other conditions,

⁸ Cf. Warburg, *Biochem. Zeitschr.*, 1921, Vol. 119, p. 134.

partly reviewed above; in addition to chemical specificity, other factors enter, the nature of which can only be surmised at present. Analogies may be pointed out between growth processes and certain forms of electro-deposition or electrosynthesis. In the electro-deposition of metals at cathodes, characteristic structure is formed which is often branching, vesicular or in other respects similar to organic structure. The precise structure of such deposits is known to depend on various factors, such as density of current, presence of other compounds in solution, *e.g.*, surface-active compounds, or hydrogen ion concentration. It is possible that the modifiability of morphogenetic processes by hormone influence, chromosomal constitution or other forms of chemical influence may depend on conditions which are related in their fundamental aspects to physical conditions of this kind—however widely the protoplasmic processes may differ in their chemical details. The degree to which such conceptions can be applied with advantage in the physiological study of morphogenesis can be determined only by future investigation.

MODIFICATION OF DEVELOPMENT IN RELATION TO DIFFERENTIAL SUSCEPTIBILITY¹

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ACCORDING to the older preformistic theories of heredity, there was, strictly speaking, no problem of development as distinct from the problem of heredity. The course of development was predetermined by the hereditary mechanism, at least for the earlier stages, and modifications were possible only as this mechanism was modified. The older epigenetic theories assumed the action of environmental factors, but were unable to present demonstrative evidence in support of their conclusions. The theories of heredity now current, however, do not include a theory of development. They tell us that every cell inherits the whole germ plasm, but they do not tell us how the cells of the multicellular organism become different in the course of development. If we suppose that these differences result from distribution to different cells of substances regionally localized in the cytoplasm of the egg, we have still to account for the origin and distribution of the substances in the egg cytoplasm. If these are determined by reaction between the genes and the cytoplasm in the egg, we should expect, since every cell inherits the whole germ plasm, that many, if not all, cells would come in course of time to resemble the egg more or less closely, even though they were more or less different as regards their cytoplasm at the time of their origin. The only possible view seems to be that regional cytoplasmic differences arise in relation to factors external to the protoplasm concerned. If this be true, the

¹Second paper of symposium of the American Society of Zoologists in conjunction with the American Society of Naturalists and the Botanical Society of America on "Morphogenesis." Read at Cincinnati, December 29, 1923.

problem of the origin, nature and significance of these cytoplasmic differences constitutes a problem of development distinct from the problem of heredity. The present paper is concerned with certain aspects of this developmental problem.

Physiological polarity and symmetry are expressions of factors in some way concerned in development with the spatial localization and order and the chronological sequence in appearance of specialized structures and functions characteristic of the individual, that is, with the general features of individual pattern. Since they are of such general significance and appear in protoplasms of widely different constitution, they must be in large measure independent of specific protoplasmic constitution. That polarity at least has been so regarded is indicated by the fact that it has often been assumed to be a fundamental property of living protoplasm.

The assumption has often been made that the physiological polarity and symmetry of organisms are based on a structure and orientation of molecular or molar particles, either analogous to that of the crystal or of unknown nature, and biological literature contains various attempts to interpret phenomena of individual development and reconstitution in such terms. These hypotheses are open to various objections: First, studies with polarized light give evidence of such structure only in certain specialized parts, not in protoplasm in general; second, if such structure exists in protoplasm we should expect it to be extremely stable and not readily altered by external conditions, but polarity and symmetry, at least in the simpler organisms, can be altered in many ways, or even obliterated and determined anew by factors which we can not readily conceive as altering such a fundamental structure; third, the results of reconstitution of isolated pieces, particularly the development of partial and bi-axial structures, can not be interpreted in terms of these hypotheses without special assumptions for the special cases; fourth, crystalline structure disappears when

chemical reaction takes place and it is difficult to conceive how such a structure of the magnitude necessary as a basis for physiological polarity and symmetry can persist in protoplasm through the series of physical and chemical changes which constitute development in its dynamic aspects and how it can control and localize these changes so that an orderly whole results.

The morphological theories of polarity and symmetry which assume a stratification or localization or directed flow of so-called formative substances, or some other morphological basis, do not account for the origin of the structure assumed and meet with many difficulties, particularly in the phenomena of reconstitution of pieces.

Living protoplasms are systems in which continuous chemical and physical change takes place in a complex substratum which affects the course of this change and is affected by it. The relation between structure and function in protoplasms appears to be somewhat analogous to that existing in a stream between the banks and bed and the flowing water. If there is any truth in this conception of protoplasm, it seems entirely reasonable to believe that dynamic factors are at least as fundamental as structural factors in polarity and symmetry.

Various lines of evidence which can be mentioned only very briefly indicate that polarity and symmetry are fundamentally associated with the dynamics of protoplasm. Graded differences in rate of oxygen consumption and carbon dioxide production demonstrated by Hyman and others and of certain oxidation-reduction reactions, *e.g.*, reduction of potassium permanganate and methylene blue and the indophenol reaction, graded differences in electric potential and in susceptibility to different chemical and physical agents, and graded differences in rate of development and growth and often in structure, all agree in indicating that a physiological axis in its simplest terms is characterized by an essentially quantitative gradation in physiological condition and that rate of metabolic reactions, particularly the physiological oxida-

tions, is an essential factor in such a gradation. This evidence constitutes the basis for the terms "axial gradient," "metabolic gradient" and "physiological gradient," which explain themselves.

The question at once arises whether these quantitative gradients are actually the physiological basis of polarity and symmetry or merely indicators or results of some more fundamental condition. The experimental evidence shows first that alteration in slope or steepness of a gradient alters localization and proportions of parts in relation to that axis, second, that experimental obliteration of a gradient obliterates the corresponding polarity or symmetry from the developmental process and there is no evidence of the persistence of anything representing the axis concerned, and third, that experimental determination of a new gradient by differential exposure to light, electricity, oxygen or any other effective factor, determines a new physiological axis, provided the external factor acts long enough to determine more or less persistent protoplasmic differences at different levels.

All the evidence at present available forces us to the conclusion that polarity and symmetry of the kind which characterizes all the higher organisms, both plant and animal, and at least a large proportion of the simpler multicellular and the unicellular forms, are primarily quantitative gradients or differentials in physiological condition and that dynamic factors are at least as fundamental as any structural factors in such gradients. This conclusion does not necessarily involve the assumption that such gradients are the basis of all orderly form in living protoplasms. Stereochemical factors, colloidal phase relations, surface tension, molar pressures and tensions, and doubtless various other factors may be concerned in determining form with definite axial relations in intracellular structure, in single cells or even in some cell aggregates. But at present it seems to be true that these factors are chiefly concerned with elements of form and structure in organisms rather than with the general

axial relations of whole organisms, though it is by no means impossible that form and axial relations in some of the simpler organisms may be in large measure or wholly determined by such factors. However this may be, there is at present no actual evidence that such factors constitute the basis of the polarity and symmetry which are expressed in the orderly localization, differentiation and functional relations of axiate organisms in general. In fact, the evidence at hand indicates very clearly that such factors do not play any fundamental rôle in the establishment and maintenance of such physiological axes, though it is conceivable that they may be concerned in one way or another in some cases. In general, the physiological axis appears to be a regional quantitative differential imposed upon the protoplasmic system from without and consisting in its simplest terms of a more or less definite physiological gradient.

It may be pointed out, however, that each such gradient is a gradient in a protoplasm of specific hereditary constitution and therefore that this specific constitution is a factor in determining the modifications which the gradient may undergo during development and its final characteristics and fate, no matter how it originated or what its primary form, slope, etc. Moreover, the particular kind of organ or part which arises at a given level of an axis is determined by the specific protoplasmic constitution in reaction with the actual physiological condition existing at that level. In hydra protoplasm, for example, the hypostome and tentacles arise at the high end of the polar gradient, in frog protoplasm a tadpole head. In both cases, secondary gradients are also concerned in the formation of these and other parts, but they are different in the two forms and develop in different ways. It may also be pointed out that a gradient or gradients once established in a protoplasm may persist through cell division or other reproductive processes and so be hereditary for the resulting individual or individuals. This is the case in many processes of fission, *e.g.*, in *Paramecium*, in

Planaria and many other forms, and may also be the case in some animal eggs. Obviously, such inheritance involves no Lamarckian assumptions.

If physiological axes are primarily quantitative physiological gradients and if they are effective factors in determining localization and proportion of parts in individual development, alteration of the gradients in early stages must result in modifying development, and experiment shows that modification and control of development in this way are possible. Differential susceptibility to the action of physical and chemical agents in certain ranges of concentration or degree of action is a characteristic feature of physiological gradients. In the earlier stages of development and in many of the simpler organisms throughout life, that is, in all cases in which specific differentiation of different parts has not proceeded too far, this differential susceptibility is non-specific for at least a large number, if not for all external agents in certain ranges of concentration or degree of action. A comparison of the data on susceptibility with those on oxygen consumption, carbon dioxide production, reduction of permanganate, the indophenol reaction and rate of growth and development, brings to light the following relation between susceptibility and physiological condition, as characteristic of the earlier stages of development and of many of the simpler organisms throughout life. Susceptibility to concentrations or degrees of action above the range of tolerance or acclimation and either lethal in course of time or producing effects not readily reversible, varies directly with, though not necessarily proportionally to the rate of metabolic reactions, as indicated by the various methods which give data on metabolic rate. On the other hand, the ability to recover after temporary exposure to a certain range of concentration or degree of action and the ability to acclimate or acquire tolerance to a certain range of relatively low concentrations or degrees of action also varies directly with, though not necessarily proportionally to, the rate of metabolic reactions as determined or indicated by other methods.

The experimental basis for these conclusions consists of data on several hundred species of animals and plants, including among animals representatives of all the larger phyla. These data, obtained by various independent methods, agree in showing or indicating that differences in susceptibility along a physiological axis are primarily non-specific for certain ranges of concentration or degree of action of external agents and depend upon quantitative differences in physiological condition in which rate of metabolic reactions is an essential factor. In the work on susceptibility the following agents have been used: potassium cyanide; various more or less anesthetic agents including ethyl alcohol, ethyl ether, chloroform, chlorotone, several of the urethanes, etc.; formaldehyde; various acids and alkalis; several alkaloids; many neutral salts, including salts of sodium, potassium, magnesium, lithium, copper, mercury and others; the vital dyes neutral red, methylene blue and Janus green; the negative condition lack of oxygen; and the physical agents, extremes of temperature, ultra-violet radiation and visible light after photochemical sensitization by means of eosin or other agents. By no means all these agents have been used on all forms examined, but in every case several from different groups have been used and it has been found that for certain ranges of concentration or degree of action, experimentally determined for each species and each agent, the susceptibility gradient representing a particular axis shows the same direction with respect to that axis for different agents, though the quantitative differences in susceptibility at different levels may differ with different agents. For the protoplasms of different species widely different concentrations or degrees of action of a particular agent are often necessary, that is to say, the susceptibility of a particular species-protoplasm to external agents in general is specific, but within the individual of the species the differences in susceptibility along an axis are primarily non-specific for different agents, at least to a very high degree.

The point must be emphasized that these differences are only primarily non-specific. As differentiation progresses, particularly in the higher animals, indications of apparent specificity of particular parts to particular agents appear and it is only among the simpler organisms that the primary non-specific differences in susceptibility persist throughout life. In highly differentiated forms such as mammals and man the susceptibilities of particular organs to particular agents appear to become specific to a high degree during the course of development.

So far as the differences in susceptibility along an axis are non-specific for different agents, the only conclusion possible is that the physiological differences on which they depend are non-specific, and if they are not specific they must be quantitative. Moreover, it has been demonstrated by other methods quite independently of work on the gradients, that quantitative physiological differences, *e.g.*, differences in rate of metabolism, do determine differences in susceptibility, and it has also been shown that quantitative differences in physiological condition are characteristic of physiological axes. In short, the evidence from susceptibility agrees with that obtained by other methods in indicating that a physiological axis is primarily a quantitative gradient involving in each case dynamic factors specific for the particular species-protoplasm and that specific regional differences along an axis arise secondarily. The evidence taken as a whole points unmistakably to a quantitative dynamic basis for polarity and symmetry and so far as I am aware, there is no actual evidence but only speculative hypotheses in support of any other conception.

By way of reply to critics, it may be noted that on the basis of the evidence non-specific differences in susceptibility along an axis can not be due merely to differences in permeability of limiting surfaces because they are similar for agents which penetrate readily through living membranes and kill by accumulation within the cells, for

agents which alter or kill the protoplasm as they penetrate and for physical agents in the action of which permeability is not concerned. Moreover, the regions most affected by high concentrations of chemical agents acclimate to or recover from the action of low concentrations most readily.

Neither can these non-specific differences in susceptibility be regarded, as some critics have assumed, as signifying that all agents act in the same way upon a given protoplasm. This is obviously not the case. Though our knowledge of the action of external agents on protoplasm is far from complete, it is sufficiently evident that different agents act in very different ways. Non-specific susceptibility apparently does not depend upon the way in which a particular agent acts, but appears to be a special case of a general relation between dynamic systems and disturbance. For present purposes this relation may be stated as follows: given a dynamic system in process of equilibration, a disturbance of any essential factor of the system adequate to produce disruption in course of time or to alter the system irreversibly will in general produce such effects more rapidly when the rate of change characteristic of the system is high than when it is low. On the other hand, when the disturbance is within the limits of tolerance or equilibration of the system, equilibration to its action, *i.e.*, acclimation, or recovery from its effects will occur more rapidly in the system with high than in that with low rate of change. These relations hold because in both cases the rate of change characteristic of the system becomes a factor in determining the effects of the disturbance upon the whole. For example, a barrier thrown across a flowing stream, a diversion channel or a filling of the bed will produce its effects, *e.g.*, formation of a lake, diversion, draining of the bed below, etc., more rapidly in the rapid than in the slow stream, and equilibration to the presence of a slight obstacle or recovery from the effects of a temporary disturbance will also occur more rapidly in the rapid than in the slow stream.

The existence of quantitative gradients in physiological condition along the axes of organisms and the non-specific differential susceptibilities of different levels of these gradients provide a physiological basis for the modification and control of morphogenesis by means of external physical and chemical agents and for the interpretation of the modifications produced. On this basis the following modifications are possible: First, with relatively high concentrations or degrees of action of inhibiting agents which must be experimentally determined for each species-protoplasm and each agent, differential inhibition is produced, the degree of inhibition of development varying with the activity and susceptibility at different levels of the axis or axes; second, with accelerating agents it is possible within certain limits to produce differential acceleration, the degree of acceleration and enlargement varying with the susceptibility at different levels; third, with continuous exposure to a certain range of low concentration or degree of action, differential acclimation is possible, the rate and degree of acclimation and therefore of change in rate of metabolism and development varying with the activity at different levels; fourth, differential recovery follows temporary exposure to certain concentrations or degrees of action, the rate and degree of recovery varying with the activity at different levels. In differential inhibition the regions of greatest activity, apical or anterior and median ventral in most bilateral invertebrates, median dorsal in vertebrates, are most inhibited and therefore least developed, but with certain degrees of differential inhibition the less active and therefore less inhibited regions show hyperplasia because, under the altered metabolic relations, they are able to obtain a larger proportion of the nutritive material available. In certain degrees of differential acceleration, acclimation and recovery the changes in form and proportion are in the opposite direction.

These modifications are not specific for different agents, and even in different species and groups they

show the same differentials with respect to the axis or axes present. In this connection it is of great interest to note that in the history of developmental physiology there are no well-established cases of developmental modification in animals resulting from uniform exposure of the whole embryo at early stages to action of an external agent, which are specific for a particular agent. Cases originally regarded as specific effects of particular agents have been shown on further work to be non-specific. For example, the exogastrula, at first supposed by Herbst to be a specific effect of lithium, can be produced by many different agents of very different constitution and method of action and is now known to be primarily a result of certain degrees of differential inhibition. Again, cyclopia in fishes, the "magnesium embryo" of Stockard, is now known not to be specific for magnesium, but to result from the action of many different agents and is likewise a result of differential inhibition. Moreover, various investigators in the past have expressed the belief that the action of external agents on development is at least not commonly specific. More than 30 years ago Dareste pointed out that the same sorts of developmental modifications were produced in the chick by various external agents. The work under discussion here is then directly in line with earlier work, but attempts to go one step further in providing on the basis of many different lines of evidence a general physiological interpretation of the observed facts.

Modification of embryonic development through differential susceptibility to external agents in relation to the physiological gradients has been studied in hydroids (Child), sea urchin, starfish and sand dollar (Child, MacArthur, Hinrichs), annelids and ascidians (Child), fishes (Gowanloch) and amphibia (Bellamy), and the data of earlier investigators are essentially contributions to the same subject. The production of non-specific modifications in reconstitucional development in *Planaria* through differential susceptibility to external agents in relation to

the physiological gradients has been a feature of laboratory class work for a number of years.

Because of the large amount of work done on modification of larval development in the sea urchins, this form serves best as a basis for concrete illustration of the directions, relation to the gradients and non-specific character for different agents of the modifications, but brief comparative reference is made to developmental modifications in the hydroid, the frog and the reconstitucional development of pieces of *Planaria*.²

In the early sea urchin blastula the gradient, as determined by susceptibility to many chemical agents, to lack of oxygen, to light, etc., and by rate of oxidation-reduction reactions, is apico-basal, the high end being apical, but as development progresses the high activity of the apical region extends along a meridian which becomes median anterior in the later larva. Consequently, in the later gastrula, the prepluteus and early pluteus activity and susceptibility decrease in all directions from apical and median anterior regions.

Exposure of the early stages to concentrations of external agents which inhibit development results first in a greater inhibition of apical as compared with basal region and later of apical and median anterior as compared with basal, lateral and posterior. All degrees of differential inhibition can be produced, ranging from forms with slightly reduced apical region (oral lobe) and small angle between the arms, through forms with parallel arms, with arms fused in the median line, to the complete obliteration of bilaterality and finally of polarity also.

Exposure to a certain range of lower concentrations or degrees of action or temporary exposure followed by return to sea water produces at first some degree of differential inhibition, but later a more rapid acclimation or recovery of the more active apical and median anterior regions occurs and with certain degrees of such differential

² In the original presentation of the paper the developmental modifications discussed were illustrated by charts.

recovery the proportions of the larva are altered in directions opposite to those characteristic of differential inhibition. Differential accelerations produced by Dr. Hinrichs with certain concentrations of caffeine resemble in form the differential acclimations and recoveries, but are usually of large size, often larger than normal, because there is no primary inhibition. In the work on the sea urchin the following agents have been used: potassium cyanide, ethyl alcohol, ether and various other anesthetics; acids, alkalies, the alkaloid caffeine, many neutral salts, including salts of mercury, copper, lithium, magnesium, sodium, etc.; extremes of temperature, ultra-violet radiation and visible light after sensitization by eosin.

The various types of modification described above do not represent individual cases selected from the experimental cultures: they are the only types of modification found. Individual differences in susceptibility of different eggs determine a certain range of modification in any culture, but after the proper ranges of concentration or degree of action are determined it is easily possible to control the modifications so that all individuals of a culture show, for example, some degree of differential inhibition or differential acclimation or differential recovery. All modifications produced thus far by all agents used fall into one of the four groups, but they differ in degree with different agents according to the rapidity with which particular agents produce their effects, the amount of difference in susceptibility at different levels of a gradient and the rapidity of acclimation and recovery. For example, exogastrulation represents a differential inhibition, but this modification is most readily produced by agents to which the differences in susceptibility at different levels of the apico-basal gradient are large. Lithium salts, so far as tested, are such agents, but some degree of exogastrulation can be produced by many, perhaps by all external agents with proper concentration and treatment.

In the hydroid only an apico-basal gradient is present

in early stages, but later a second gradient, opposite in direction to the first, arises at the basal end of the planula. This new gradient represents a process of budding and gives rise to the first hydranth. As in the sea urchin, exposure to inhibiting agents in the earlier stages of hydroid development results in a greater degree of inhibition of apical than of basal regions and the latter show hyperplasia when the inhibiting action is not too great. This basal hyperplasia often gives rise to forms somewhat similar to exogastrulae. More extreme degrees of differential inhibition obliterate polarity completely, and the larva remains or becomes spherical and never develops any indications of an axis, though it may live for a long time.

As regards later stages, certain degrees of differential inhibition transform the hydranth-stem axis into a creeping stolon, and certain degrees of differential acclimation and recovery transform stolons into hydranth and stem. In differential acclimation or recovery of the planula, if polarity has not been completely obliterated, outgrowth may occur at one or both ends of the planula, according as one or both gradients are present. Such outgrowth is usually at first a stolon, but later may transform into hydranth and stem if the degree of acclimation or recovery is sufficient. When polarity is completely obliterated through differential inhibition, return to sea water is usually followed by the determination of one or more new gradients and axes in consequence of the differences in condition of cells on the free surface and those on the surface in contact as the cell mass lies on the bottom or in consequence of slight local differences in different regions of the mass ("adventitious" polarities). All these modifications have been produced by potassium cyanide, increase in hydrogen ion concentration, lithium chloride and neutral red and the same modifications have been observed in several species. In some species development in standing water or in water with a slightly increased carbon dioxide content or insufficient aeration produces

differential inhibition. No other modifications than those mentioned have been observed in any case.

In the frog an apico-basal gradient is characteristic of the earlier stages, as Bellamy has shown, but as gastrulation approaches the dorsal lip region becomes highly active and after closure of the blastopore the regions of highest activity and susceptibility are anterior and median dorsal, with a secondary gradient arising posteriorly as the tail bud appears. The action of inhibiting agents on early cleavage stages results in greater inhibition of apical than of basal regions and accelerating agents accelerate apical more than basal regions. In later stages, after the dorsal lip region has become active, inhibiting agents inhibit this region more than others, the form of the larva depending on the stage and period of exposure and the concentration or degree of action of the agent. Permanent yolk plugs, spina bifida, equatorial gastrulation and obliteration of bilateral symmetry all represent various degrees of differential inhibition. Still later stages show all degrees of microcephaly to almost complete acephaly, the tail is more or less inhibited and grows dorso-posteriorly instead of posteriorly because of inhibition of the dorsal region. In relation to bilaterality differential inhibition results, according to its degree in approximation to the median line of lateral organs, *e.g.*, ventral suckers, nasal primordia, eyes or in formation of a single organ in the median line, or in still more extreme cases in complete absence of these organs.

In differential acclimation and differential recovery some degree of differential inhibition in the earlier stages is followed by the more rapid acclimation or recovery of the more active regions, so that the later stages show modification opposite in direction to the results of differential inhibition. Differential acceleration produces megacephalic forms with eyes and other lateral organs far apart and with long tails.

In the work on the frog the following agents were used: potassium cyanide, formaldehyde, potassium per-

manganate, mercuric chloride, lithium chloride, hydrochloric acid, sodium hydroxide and ethyl alcohol, and the types of modification are similar to those obtained by various other investigators with some of these and with other agents. As regards relations to the axial gradients, the modifications are not specific for the different agents, but their character depends on concentration of agent, period and stage of exposure and difference in susceptibility to different agents of different levels of the gradients.

Mr. Gowanloch has obtained essentially similar series of modifications in fishes with a large number of agents, but his work is not yet published. In passing, attention may be called again to the fact that cyclopia, which Stockard formerly regarded as a specific effect of magnesium on the fish embryo, is simply a result of differential susceptibility between median and lateral regions of the head and can be produced in fishes, amphibia and many other forms by many different agents. Stockard's later interpretation of cyclopia and related modifications is along the same lines as my own, except that he seems to regard rate of development as something fundamental in itself. The evidence indicates, however, that rate of development is dependent on rate of metabolism or of certain metabolic reactions.

In the reconstitution of pieces of *Planaria* various types of anterior end, normal, teratophthalmic, teratomorphic, anophthalmic and acephalic appear. Under the usual conditions the frequency of the various types depends on physiological conditions in the piece, but all the types can be produced by all chemical and physical agents used thus far and various lines of evidence show that they represent different degrees of differential inhibition, resulting from the differential susceptibility of median and lateral regions. All degrees of approximation of the eyes, even to cyclopia and to anophthalmia, occur according to the degree of differential inhibition. On the other hand, under conditions which permit differ-

ential acclimation or differential recovery the median region of the head may show considerable overgrowth and heads which at first possess only a single median eye may later develop a right and left eye in addition. These modifications of the head in *Planaria* have been produced with potassium cyanide, various anesthetics, acids, alkalis, caffeine and extremes of temperature. They show no indication of specificity for particular agents, and their relation to the gradients is the same as in other forms. Cyclopia in *Planaria*, for example, is no more specific for any particular agent than in fishes or in amphibia, and it is the same sort of modification physiologically as the fusion in the median line of the two anal arms of the sea urchin larva.

Many other data might be presented, but these are perhaps sufficient to indicate the importance of differential susceptibility in modification of development in widely different forms and the relations between differential susceptibility and the axial gradients. The evidence forces us to the conclusion that the primary physiological differences at different levels of an axis are quantitative differences in which the metabolism characteristic of the species is a fundamental factor and that the specific differentiations of different regions and the proportions of the individual are secondary results of these differences.

ON THE RELATIONSHIP BETWEEN STATURE AND THE LENGTH OF THE APPENDAGES IN MAN

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THE change in the relative length of the trunk and the appendages during the development of the individual is a phenomenon quite familiar to anthropologists. While numerical illustrations and graphical representations of the changes in the relative proportions of the head, trunk and lower limbs with growth are to be found in many works on anthropology, the problem of the proportionality of the various parts of the body does not seem to have received the amount, or at least the kind, of attention which it deserves. The investigation of the problem can and should be carried much further. It seems important in its relationship to the physiology of development to determine whether *at any given age* the physical proportions of the individuals of a population are such as might have arisen from a retardation or cessation of growth at varying antecedent stages of development. In short, the analysis must not be limited to the comparison of average dimensions of individuals of different ages, but should be extended to groups of individuals of the same age.

The interest of the problem is not limited to its relation to morphogenesis merely. As shown by the classical studies of Galton and Pearson, stature and other dimensional characters are inherited. Castle (1914) has taken up experimentally and statistically the problem of the extent to which the inheritance of the size of the different parts of the individual is due to general growth factors. Davenport (1917) concludes that there is evidence that the elements of stature are to a certain extent separately inheritable, and further that the inheritance of the pro-

portional lengths of the elements of stature is as evident as the inheritance of the absolute dimensions. In its relation to the problem of inheritance it is therefore desirable to determine whether the proportionality of the parts at any given stage of development is dependent upon their absolute size.

Failure to have considered these problems is perhaps largely due to the fact that extensive series of data and refined quantitative methods of analysis are essential prerequisites. As far as I am aware the most extensive data on the relationship between the total height of the individuals and the amount contributed to this total by one of the elements of stature in a relatively homogeneous series of individuals are the series of correlations between total stature and sitting height in school children of various ages published by Boas and Wissler (1906) and the more recent determination of the correlations between stature and total leg and thigh length in Oxford students due to Schuster (1911).

But stature as a whole is made up of head and trunk length and leg length. On *a priori* grounds one would, therefore, have expected the correlations found by Boas and Wissler and by Schuster to be high. Those published by Boas and Wissler for stature and sitting height are of the order .7-.9. Those measuring the closeness of correlation between stature and leg and thigh length in Oxford students are of the order .8 for the thigh and .9 for the whole leg.

Since common observation and the diagrams showing the change in the proportionality of the elements of stature in text-books on anthropology show that in the later stages of development the greatest source of increase in stature is the growth of the lower limbs, it is difficult to see why anthropologists have taken sitting height (used as a measure of trunk + head length) rather than leg length, which may *for purposes of rough approximation* be taken as the difference between total height and sitting height, in determining their correlations.

While the published data are given in the form to determine the correlation between total stature and sitting height, it is quite possible to determine the correlations for total stature and leg length (Harris, 1917). Since our primary purpose is not a discussion of the correlation between absolute dimensions these coefficients will not be discussed here.

While the coefficients showing the relationship between stature and sitting height or stature and leg length as deduced by Boas and Wissler, Schuster and others are of great value in that they express a relationship which had heretofore been merely a vague conception on a universally comparable quantitative scale, they fail to give all (and, indeed, the most important) information concerning the interrelationship of these variables.

The first requisite for progress in the analytical as compared with the purely descriptive treatment of such problems seems to be a formula which will measure the relationship between total stature and the proportional length of any of its constituents.

It is obvious that tall children or adults have on the average longer legs than short ones. Do they have relatively longer legs in populations at each stage of development?

Schuster (1911), in determining the correlation between stature and the ratio $\frac{\text{length of leg}}{\text{stature}}$ has recognized the need of such analysis. His results will be noted below.

I believe the correct method for dealing with all such problems to be that worked out several years ago by Professor Pearson and myself and since then rather widely applied to biological problems (Harris 1909, 1918).

This coefficient measures the relationship between a variable, such as stature, and the deviation of a dependent variable, such as sitting height, from its probable value on the assumption that the two variables should bear the same proportional relationship to each other

throughout their ranges of variation, *i.e.*, that in any individual case, $s = ph$ where $p = \bar{s}/\bar{h}$, the bars denoting the means of the independent variable h = height, and of the dependent variable s , = sitting height; s' is the theoretical value of s , and the correlation between h and z , = $s - s'$, or the deviation of sitting height from its probable value, shows whether s becomes relatively larger or smaller with variations of h above or below the population mean.

A convenient working formula is

$$r_{hz} = \frac{r_{hs} - \bar{V}_h / \bar{V}_s}{1 - r_{hs}^2 + (\bar{V}_h / \bar{V}_s)^2}$$

where $V_h = \sigma_h / h$, $V_s = \sigma_s / s$, the sigmas denoting standard deviations and the bars the means of the two characters.

In working with the data of Boas and Wissler we have the advantage of dealing with the individuals in a number of different age groups, characterized by the increasing dimensions of each of the characters. The great regularity of the increase in the average dimensions of the subjects is shown in diagram 1 which represents the change in the measurements of Worcester boys with increasing age. The straight lines are fitted to the means. Probably the irregularity in the rate of increase in total stature noticeable at 14 to 16 years is due to the onset of puberty.

The curve for growth in stature found by Bowditch for Boston school children and reproduced by Minot (1908) indicates essential linearity until the age of 12 to 14 years when the onset of puberty introduced disturbing factors. The same is true of the growth of Berlin school children as illustrated by Martin.

From Table Ia and Ib of Boas and Wissler we abstract the means and standard deviations of stature and sitting height in Worcester boys and girls for each year of age from 6 to 17. The correlations between stature and sitting height deduced by Boas and Wissler are given in their Tables IIa and IIb. Inserting these values in our

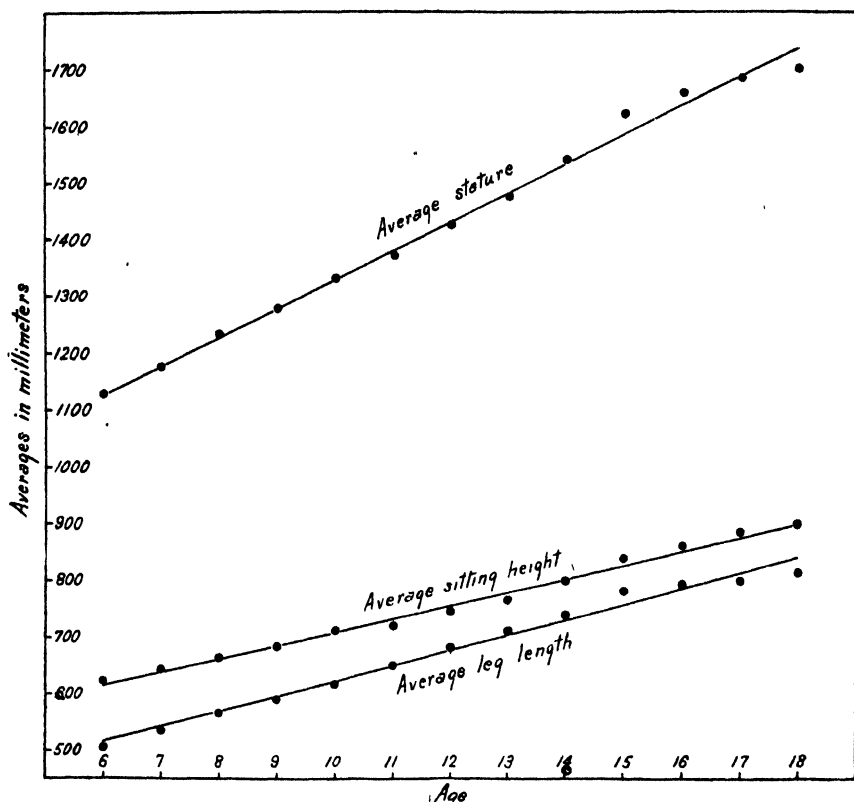


DIAGRAM 1. Regression of sitting height, leg length and stature on age in Worcester boys.

formula, we obtain the correlations between stature and the deviation of sitting height from its probable value. These are laid beside the correlations between stature and sitting height published by Boas and Wissler in table I. Without exception the coefficients are negative in sign. They range from $-.13$ to $-.47$, with a general average of $-.256$ for boys, and from $-.10$ to $-.48$ with a general average of $-.292$ for girls.¹

The constants for more extensive data for Milwaukee and Toronto boys and girls may be deduced from Tables VIa, VIb and VIIa of Boas and Wissler. The results in Table II fully confirm the conclusions drawn from Table I.

¹ These averages are obtained without weighting. If the contestants be weighted with the number of children upon which they are based the results are essentially the same, *i.e.*, $-.258$ for boys and $-.276$ for girls.

Unfortunately, Boas and Wissler have not recorded their statistical constants to enough places to give smooth results when they are used for further calculations.

TABLE I
RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT IN WORCESTER
BOYS AND GIRLS

Age	Boys				Girls			
	<i>N</i>	Correlation between stature and sitting height	Correlation between stature and deviation of sitting height	$\frac{r}{E_r}$	<i>N</i>	Correlation between stature and sitting height	Correlation between stature and deviation of sitting height	$\frac{r}{E_r}$
6	110	.78 ± .03	— .47 ± .05	9.4	99	.67 ± .04	— .46 ± .05	9.2
7	203	.81 ± .02	— .22 ± .05	4.4	129	.74 ± .03	— .42 ± .05	8.4
8	187	.83 ± .02	— .37 ± .04	9.3	146	.81 ± .02	— .32 ± .05	6.4
9	225	.79 ± .02	— .29 ± .04	7.3	152	.81 ± .02	— .35 ± .05	7.0
10	228	.80 ± .02	— .33 ± .04	8.3	174	.84 ± .02	— .30 ± .05	6.0
11	211	.88 ± .01	— .13 ± .05	2.6	202	.83 ± .02	— .19 ± .05	3.8
12	256	.88 ± .01	— .15 ± .04	3.8	208	.84 ± .01	— .31 ± .04	7.8
13	224	.90 ± .01	— .27 ± .04	6.8	190	.80 ± .02	— .10 ± .05	2.0
14	228	.92 ± .01	— .30 ± .04	7.5	151	.85 ± .02	— .19 ± .05	3.8
15	183	.85 ± .01	— .17 ± .05	3.4	138	.81 ± .02	— .12 ± .06	2.0
16	116	.79 ± .02	— .27 ± .06	4.5	101	.71 ± .03	— .48 ± .05	9.6
17	51	.77 ± .04	— .18 ± .09	2.0	69	.71 ± .04	— .26 ± .08	3.3

TABLE II
RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT IN MILWAUKEE AND
TORONTO BOYS AND GIRLS

Age*	Boys				Girls			
	<i>N</i>	Correlation between stature and sitting height	Correlation between stature and deviation of sitting height	$\frac{r}{E_r}$	<i>N</i>	Correlation between stature and sitting height	Correlation between stature and deviation of sitting height	$\frac{r}{E_r}$
M. 5	296	.73 ± .02	— .32 ± .04	8.0	250	.77 ± .02	— .34 ± .04	8.5
T. 7	910	.76 ± .01	— .23 ± .02	11.5	800	.73 ± .01	— .25 ± .02	12.5
M. 7	641	.84 ± .01	— .33 ± .02	16.5	586	.85 ± .01	— .25 ± .03	8.3
T. 10	853	.78 ± .01	— .36 ± .02	18.0	848	.79 ± .01	— .22 ± .02	11.0
M. 10	553	.80 ± .01	— .42 ± .02	21.0	553	.93 ± .00	— .16 ± .03	5.3
T. 13	600	.80 ± .01	— .41 ± .02	20.5	675	.72 ± .01	— .10 ± .03	3.3
M. 13	365	.95 ± .00	— .16 ± .03	5.3	333	.85 ± .01	— .35 ± .03	11.7
M. 16	58	.83 ± .03	— .05 ± .09	0.6	108	.62 ± .04	— .09 ± .06	1.5

* M = Milwaukee, T = Toronto.

Furthermore, the number of individuals upon which their constants for total stature and sitting height are based sometimes differs. This introduces another possible source of error. While the consistency of the end results in Tables I and II is such as to make it practically certain that these sources of error are of little practical importance, I have thought it worth while to draw from their tables of actual measurements of boys (pages 49-132) a series of individuals in which the record of stature, sitting height and weight are all included.² The results of calculations based on these original measurements are given in Table III.

TABLE III

RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT IN WORCESTER BOYS

Age	N	Total height and sitting height	$\frac{r}{E_r}$	Total height and deviation of sitting height from its probable value	$\frac{r}{E_r}$
6	104	.86 \pm .02	48.3	— .25 \pm .06	4.1
7	144	.74 \pm .03	29.0	— .27 \pm .05	5.2
8	111	.85 \pm .02	47.1	— .24 \pm .06	4.0
9	133	.79 \pm .02	36.1	— .27 \pm .05	4.9
10	122	.82 \pm .02	41.8	— .32 \pm .05	5.8
11	113	.80 \pm .02	35.1	— .39 \pm .05	7.4
12	147	.89 \pm .01	76.0	— .22 \pm .05	4.1
13	129	.86 \pm .02	56.3	— .38 \pm .05	7.4
14	128	.91 \pm .01	85.6	— .19 \pm .06	3.3
15	140	.92 \pm .01	105.7	— .10 \pm .06	1.8
16	92	.78 \pm .03	28.1	— .29 \pm .06	4.5
17	47	.78 \pm .04	20.0	— .31 \pm .09	3.5
18	48	.85 \pm .03	30.9	— .16 \pm .09	1.7

The values are in excellent general agreement with those based on the constants of Boas and Wissler.

To test the influence of age heterogeneity at this period of relatively rapid development upon the relationship under investigation we have grouped the series of data upon which the results in Table III have been based into two-year classes. The coefficients are given in Table IV.

The results show that the correlation between stature and sitting height for two-year groups lies between that

² The records in the table of repeated measurements were not included. Unfortunately, there are evidences of typographical errors in these protocols which cast some doubt upon the exactness of constants based upon them.

for the single year periods in the earlier years, but is larger than that of either of the two years in the constants determined for 14-17 years. Whether this result is generally valid or whether it is a peculiarity of these series of data can not be asserted without further work.

In the correlation between stature and the deviation of sitting height from its probable value the coefficients in the earlier pairs of years are generally larger than either of the two constituent years. In the later pairs of years they are intermediate.

TABLE IV .

RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT IN WORCESTER BOYS
CLASSIFIED IN TWO-YEAR AGE GROUPS

Age	N	Total height and sitting height	r $\frac{r}{E_r}$	Total height and deviation of sitting height from its probable value	r $\frac{r}{E_r}$
6 and 7	248	.82 \pm .01	82.0	— .28 \pm .04	7.0
8 and 9	244	.83 \pm .01	83.0	— .29 \pm .04	7.3
10 and 11	235	.80 \pm .02	80.0	— .42 \pm .04	10.5
12 and 13	276	.88 \pm .01	88.0	— .33 \pm .04	8.3
14 and 15	268	.93 \pm .01	93.0	— .14 \pm .04	3.5
16 and 17	187	.82 \pm .02	41.0	— .20 \pm .05	4.0

Since the values of the correlations between stature and the deviation of sitting height from its probable value are not very greatly different in groups of one year and of two year age range, it is evident that this relationship is largely due to differentiation occurring earlier in life than the age groups covered by these data, and not primarily to age heterogeneity within the groups under investigation.

As a further test of the validity of the conclusions here drawn I have determined the relationships on small groups of individuals selected from the series of Boas and Wissler because measurements of forearm, to be discussed later, were also available.³

The essential constants set forth in Table V are fully confirmatory of those already given.

³ These are children given in their repeated measurement tables.

Schuster's correlations are based on stature and leg length, not on stature and sitting height. His constants and those which have been deduced from them are laid side by side in Table VI.

TABLE V
RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT IN WORCESTER BOYS

Age	Number	Correlation between stature and sitting height	Correlation between stature and deviation of sitting height from its probable value
7	42	.86 \pm .03	— .43 \pm .09
8	63	.80 \pm .03	— .32 \pm .08
9	67	.73 \pm .04	— .08 \pm .08
10	81	.81 \pm .03	— .30 \pm .07
11	72	.79 \pm .03	— .15 \pm .08
12	71	.87 \pm .02	— .38 \pm .07
13	76	.87 \pm .02	— .07 \pm .08
14	60	.84 \pm .03	— .32 \pm .08

TABLE VI
RELATIONSHIP BETWEEN STATURE AND LENGTH OF LOWER APPENDAGES IN OXFORD STUDENTS

Age	Stature and leg r_{hl}	Stature and thigh r_{ht}	$r_{hl}-r_{ht}$	Stature and deviation of leg r_{hz_l}	Stature and deviation of thigh r_{hz_t}	$r_{hz_l}-r_{hz_t}$
18	.87 \pm .01	.80 \pm .02	+ .07 \pm .02	.42 \pm .05	.47 \pm .04	— .05 \pm .06
19	.86 \pm .01	.74 \pm .02	+ .12 \pm .02	.35 \pm .03	.26 \pm .03	+ .09 \pm .04
20	.89 \pm .01	.82 \pm .02	+ .07 \pm .02	.39 \pm .04	.36 \pm .04	+ .03 \pm .06
21	.91 \pm .01	.82 \pm .02	+ .11 \pm .02	.48 \pm .04	.34 \pm .05	+ .14 \pm .06
22	.91 \pm .01	.83 \pm .02	+ .11 \pm .02	.40 \pm .06	.34 \pm .06	+ .06 \pm .09

Note that for both stature and length of leg and stature and length of thigh the correlations are high. Those for stature and length of leg are uniformly higher than those for stature and length of thigh, just as one might expect them to be on morphological grounds.

Since the correlations for stature and the deviation of sitting height from its probable value is negative, one would expect the correlation between stature and the deviation of leg length from its probable value to be posi-

tive. This is found to be the case. These coefficients are about half as large as those given by Schuster for the relationship between the actual values of the same variables. Thus taller individuals have relatively as well as absolutely longer legs and thighs than short ones.

It is of interest to compare the correlation between stature and the deviation of leg length, z_l , and thigh length, z_t . Except for young men of 18 years the correlation between stature and the deviation of total leg length from its probable value is higher than that between stature and the deviation of thigh length from its probable value.

As noted above, Schuster has investigated the same relationship for total leg length by determining the correlation between stature and the ratio $\frac{\text{length of leg}}{\text{stature}}$. His results may be placed beside our own.

Stature and ratio		Stature and deviation	
Age 1844 \pm .05		.42 \pm .05
Age 1937 \pm .03		.35 \pm .03
Age 2042 \pm .04		.39 \pm .04
Age 2150 \pm .04		.48 \pm .04
Age 2245 \pm .06		.40 \pm .06

Uniformly $r_{hr} > r_{hz_l}$. A discussion of the whole problem would lead us quite too far into the question of correlation of indices.

The relationship between stature and sitting height will be more clearly brought out by representing the increase in the absolute sitting height and the decrease in the relative sitting height with increase in stature by straight line equations.

Utilizing the series combined in two year age groups in order to have larger numbers of individuals upon which to base averages, we have the equations and the empirical mean sitting heights represented graphically in diagram 2. This shows that the increase in sitting height with increase in stature may be excellently represented by the slope of a straight line.

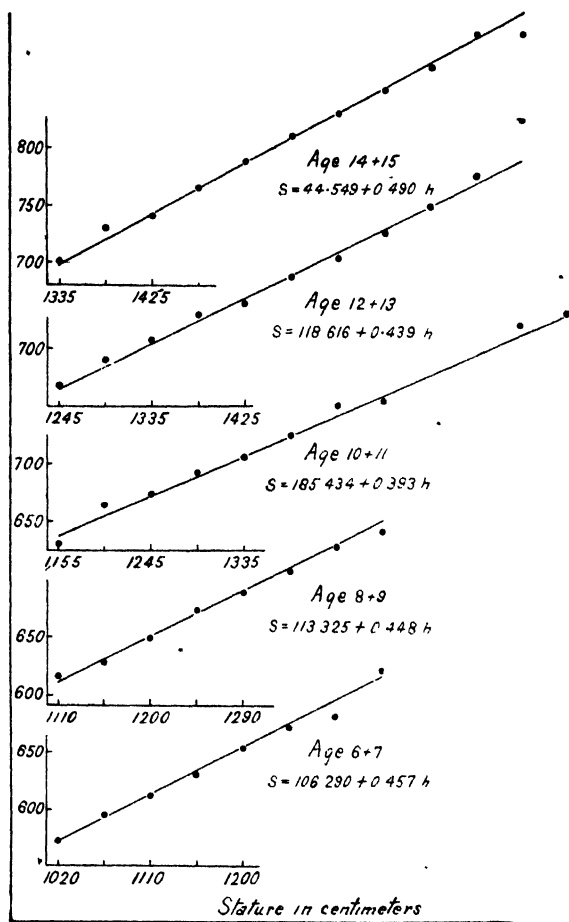


DIAGRAM 2. Regression of sitting height (ordinates) on stature in Worcester boys.

The equations showing the relationship between stature and the deviation of sitting height from its probable value and between stature and the deviation of leg length ($=h - s$, approximately) from its probable value are interchangeable with changes of sign only. Those for the regression of the deviation of sitting height from its probable value on stature are:

Ages 6 and 7	$z = 108.890 - 0.094 h$
Ages 8 and 9	$z = 106.068 - 0.084 h$
Ages 10 and 11	$z = 186.971 - 0.138 h$
Ages 12 and 13	$z = 113.466 - 0.078 h$
Ages 14 and 15	$z = 43.301 - 0.027 h$

The equations for the regression of the deviation of leg length from its probable value on stature are given and are represented graphically on Diagram 3.

These lines, showing the theoretical mean deviation of the leg lengths of individuals of different statures from

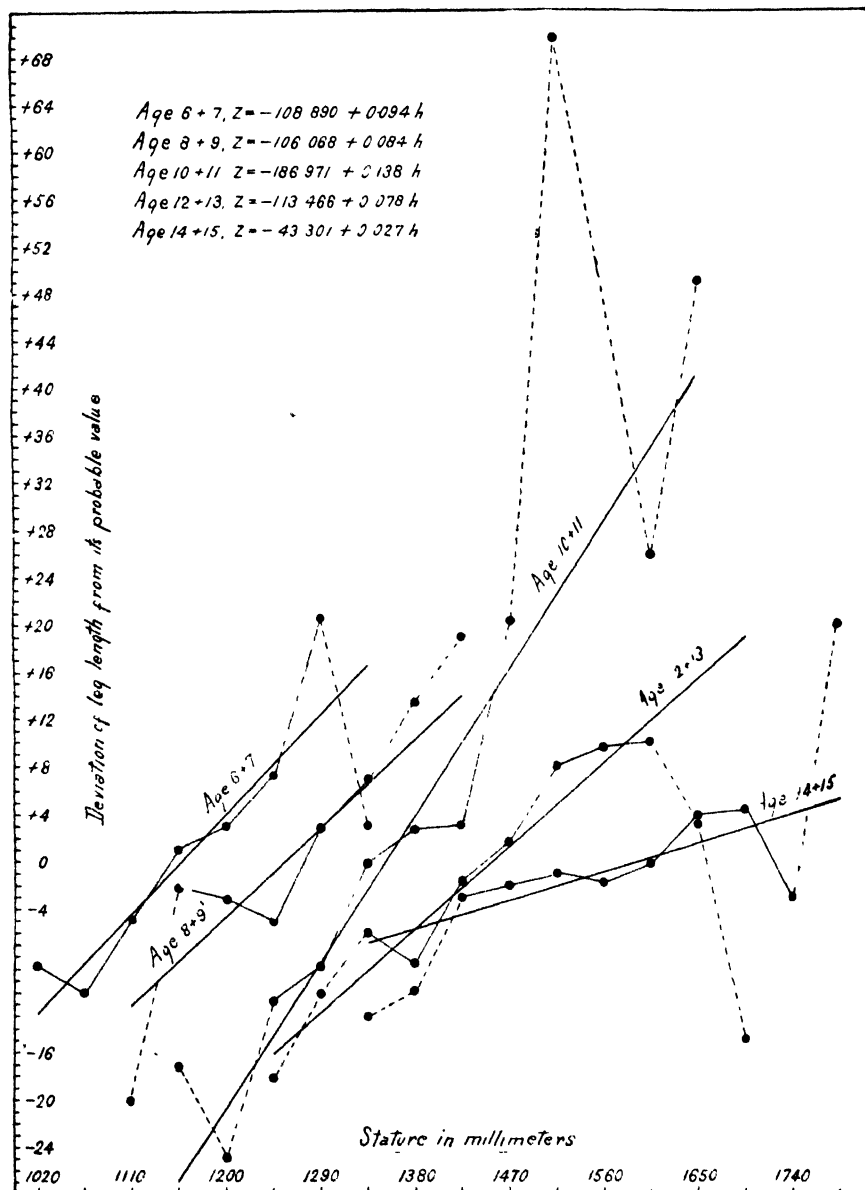


DIAGRAM 3. Regression of the deviation of leg length from its probable value on stature in Worcester boys.

their probable value, show a decrease in the relative length of sitting height and an increase in the relative leg length with increase in stature in groups of individuals of two year age range.

The empirical means are very widely scattered about the lines, especially in the case of the stature groups in which the number of observations is small.

The general agreement of the lines and means is perhaps as good as might be expected from the available data.

While the formula here employed to measure the relationship between total stature and the deviation of the length of the lower appendages from their probable value has heretofore been limited to cases in which y is some fraction of x it seems quite legitimate to extend its application to at least some cases in which y is not an actual constituent part of x but considered merely in its relation to x . If this be legitimate, we may consider the relationship between stature and the length of the upper as well as of the lower appendages.

We may first consider the correlations between stature and forearm, c , given by Pearson (1903).

We find the following values:

	Correlation between Stature and Forearm r_{hc}	Correlation, Stature and Deviation of Forearm from its probable value, r_{hz_c}
Father .	.642 \pm .012	— .149 \pm .020
Son686 \pm .011	— .086 \pm .020
Mother597 \pm .013	— .171 \pm .020
Daughter704 \pm .009	— .071 \pm .018

Turning to Macdonell's (1902) data for 3000 non-habitual criminals from Scotland yard we find:

For stature and cubit, c .

$$r_{hc} = .800 \pm .004$$

For stature and deviation of cubit from its probable value

$$r_{hz_c} = - .149 \pm .012$$

Turning to Orensteen's (1915) measurements of Cairo-born natives, I have deduced from his Table XIV,

For stature and left cubit,

$$r_{hc} = .772 \pm .010$$

For stature and deviation of left cubit from its probable length,

$$r_{hz_c} = .002 \pm .024$$

Summarizing these results for stature and length of cubit in adults, we note that while all the correlations between stature and length of forearm are of the order $r = .60$ to $r = .80$, the correlations between stature and the deviation of the length of cubit from its probable value are low. Five of the six coefficients are negative in sign. These five are from about four to nearly ten times as large as their probable errors and may be reasonably regarded as statistically significant. The single positive constant is smaller than its probable error.

Expressing the change in the relative length of the forearm in terms of regression, we have the results for two of Pearson's series represented with the actual mean deviations of forearm length from its probable value in diagram 4.

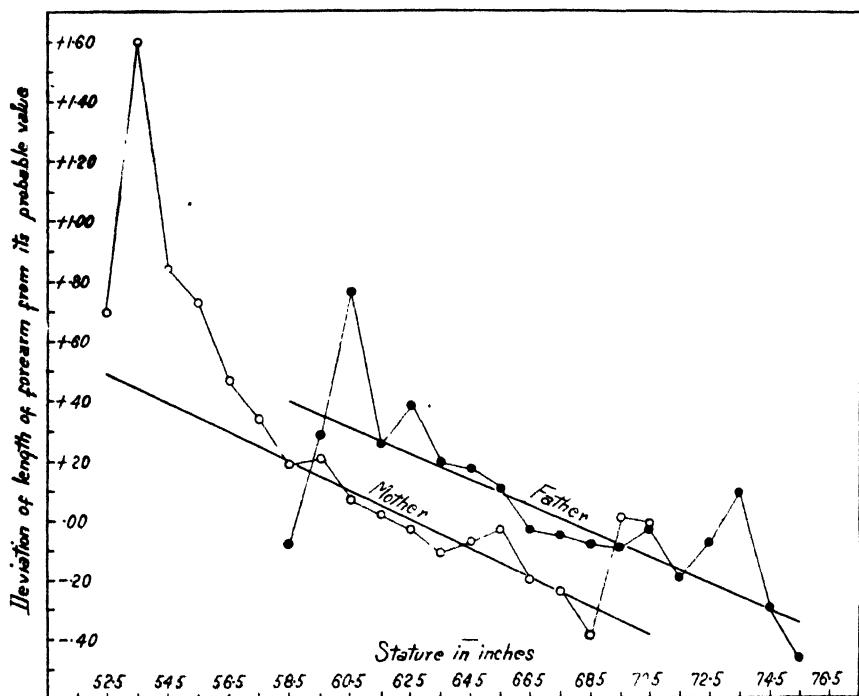


DIAGRAM 4. Regression of deviation of length of forearm from its probable value on stature in Pearson's series of adults.

Turning to the small groups of children for which Boas and Wissler have given data for length of forearm, we have the computed values for the interrelationship of sitting height and length of forearm and for stature and length of forearm given in Table VII.

The great irregularity of the results is perhaps largely due to the small numbers of children available and the untrustworthiness of some of the published measurements, as already indicated.

It is to be noted first of all that the correlations between stature and forearm are higher than those between sitting height and forearm. This means merely that prediction of forearm can be made more exactly from the length of the trunk and the length of the leg than from one of these dimensions alone.

TABLE VII

RELATIONSHIP BETWEEN STATURE AND SITTING HEIGHT AND THE LENGTH OF THE APPENDAGES IN WORCESTER BOYS

Age	Number	Correlation for sitting height		Correlation for stature	
		Sitting height and forearm	Sitting height and deviation of forearm from its probable value	Stature and forearm	Stature and deviation of forearm from its probable value
7	42	.69 \pm .05	+ .21 \pm .10	.77 \pm .04	+ .27 \pm .10
8	63	.53 \pm .06	— .10 \pm .08	.58 \pm .06	— .03 \pm .08
9	67	.63 \pm .05	— .65 \pm .05	.83 \pm .03	— .31 \pm .07
10	81	.54 \pm .05	— .11 \pm .07	.71 \pm .04	+ .12 \pm .07
11	72	.50 \pm .06	— .34 \pm .07	.72 \pm .04	+ .00 \pm .08
12	71	.76 \pm .03	— .15 \pm .08	.87 \pm .02	— .11 \pm .08
13	76	.71 \pm .04	— .10 \pm .08	.84 \pm .02	+ .24 \pm .07
14	60	.69 \pm .04	— .15 \pm .09	.87 \pm .02	+ .11 \pm .09

The coefficients showing the relationship between sitting height and the deviation of forearm length from its probable value are with one exception negative in sign, indicating that shorter forearm is associated with greater trunk and head length. The values for the correlation between stature and the deviation of forearm length from its probable value are in part positive in sign. They do not justify any conclusion concerning the variations in

the relative length of the forearm with variation in stature other than that the relationship, if it exists at all, is very small.

Table V, which gives the relationship between stature and the deviation of sitting height from its probable value in the same series of individuals, shows that the latter relationships are clearly significant. It is evident, therefore, that the relationships between stature and the upper and lower appendages are of a very different nature.

Summarizing the results of all the data for length of forearm we may conclude that if there be any relationship at all between stature and the relative length of the forearm it is of a negative kind, relatively shorter forearm being associated with greater stature. Thus the dimensions of the upper and lower appendages show a quite different relationship to total stature.

Macdonell (1902) has also given data for the relationship between stature and the length of the left middle finger. The results are:

For stature and length of left middle finger, *f*,

$$r_{hf} = 0.661 \pm 0.007$$

For stature and deviation of length of left middle finger from its probable value,

$$r_{hzf} = -0.204 \pm 0.012$$

We may also utilize the 802 measurements of stature and left middle finger in Cairo-born natives given by Orensteen (1915).

The results deduced from his Table XI are:

For stature and length of left middle finger,

$$r_{hf} = 0.637 \pm 0.014$$

For stature and deviation of left middle finger from its probable length,

$$r_{hzf} = -0.037 \pm 0.024$$

SUMMARY

The purpose of this paper has been to consider the relationship between stature and the length of the upper and lower appendages in man. The data represent both children and adults of both sexes.

The correlation between stature and the length of the appendages is fairly high. In the case of the lower appendages this relationship is the inevitable result of the fact that the appendages themselves are one of the chief components of stature.

While the coefficient of correlation measuring this relationship has descriptive value, it fails to bring out fully the morphological relationship between these variables. A coefficient showing the relationship between stature and the deviation of the components (sitting height, leg length or thigh length) from their probable value shows that in tall individuals sitting height forms a relatively smaller and leg length a relatively larger proportion of total stature than in short ones. This relationship holds for both adults and children of various ages.

The relationship between stature and the deviation of the length of the upper appendages from their probable value is relatively small and in some series of data can not be considered significant in comparison with its probable error. The coefficients for the anterior appendages are prevaillingly negative in sign.

Thus anterior and posterior appendages show quite different relations to total stature.

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ABERRANT ENDOSPERM DEVELOPMENT AS A MEANS OF DISTINGUISHING LINKAGE GROUPS IN MAIZE¹

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THE determination of linkage groups in maize is attended with difficulties not met in *Drosophila*. Of these, the large number of Chromosomes (ten), the relatively few genes whose *loci* have been established and the long developmental cycle—only one or at least two generations can be grown in a year—are not without importance. But the greatest difficulty comes from the fact that crossing over occurs with approximately the same frequency in both microspore and megaspore development. (Emerson and Hutchison, 1921; Eyster, 1922). Where, as in *Drosophila*, there is no crossing over in the male, linkage, even of genes widely separated on a chromosome, can be determined readily. With maize, however, very loose linkages can not be positively identified as such until one or more other genes with intermediate loci are discovered. Since nothing is as yet known as to the length of maize chromosomes in terms of crossover units, it follows that some of the linkage groups which are now regarded as distinct may in reality pertain to widely separated regions of a single chromosome.

A method which, I am inclined to believe, affords critical evidence for the separation of certain linkage groups in maize has been found through studies of aberrant endosperm development. In an earlier paper (Emerson, 1921) it was shown that, with seeds which are heterozygous for the linked genes *C c* for Aleurone color and *Wx wx* for endosperm texture, spots of colorless aleurone are in the great majority of cases underlaid by waxy endosperm, whereas spots of colored aleurone are underlaid by starchy endosperm. This relation was found not to hold for aleurone and endosperm genes that were not linked. Thus for *A a* and *Wx wx*, which ordinary genetic tests had indicated were independently inherited, the colored and colorless aleurone spots were underlaid by starchy endosperm. It was concluded,

¹ Paper No. 123. Department of Plant Breeding, Cornell University, Ithaca, New York.

therefore, that such mosaic seeds were due to some aberrant chromosome behavior such as non-disjunction or elimination.

Since the publication of my earlier paper, additional evidence bearing on the problem has been obtained. All the available data with which I am familiar are given in Table I.

TABLE I
ABERRANT SEEDS OF MAIZE INVOLVING LINKED GENES

Item No.	Genotypes of parents		Phenotypes of mosaic seeds		Number of Mosaic seeds	Citation of published data, and pedigree number for new data
	Female	Male	Aberrant part	Normal part		
1	<i>c wx</i>	<i>C Wx</i>	<i>c wx</i>	<i>C Wx</i>	1	Collins 1913
2	<i>c wx</i>	<i>C Wx</i>	<i>c wx</i>	<i>C Wx</i>	55	Emerson 1921, table 1
3	<i>c wx</i>	<i>C Wx</i>	<i>c wx</i>	<i>C Wx</i>	3	Emerson 1921, pp. 414
4	<i>c wx</i>	<i>C Wx</i>	<i>c wx</i>	<i>C Wx</i>	8	11453, 11454, 12293
5	<i>c sh</i>	<i>C Sh</i>	<i>c sh</i>	<i>C Sh</i>	1	Emerson 1921, pp. 415, 416
6	<i>c sh wx</i>	<i>C Sh Wx</i>	<i>c sh wx</i>	<i>C Sh Wx</i>	2	11454, 12293
7	<i>i sh</i>	<i>I Sh</i>	<i>i sh</i>	<i>I Sh</i>	7	11450
8	<i>i wx</i>	<i>I Wx</i>	<i>i wx</i>	<i>I Wx</i>	1	Collins 1913
9	<i>i wx</i>	<i>I Wx</i>	<i>i wx</i>	<i>I Wx</i>	43	11451, 11452
Total					121	
10	<i>i wx</i>	<i>I Wx</i>	<i>i wx + i Wx</i>	<i>I Wx</i>	1	11452
11	<i>i wx</i>	<i>I Wx</i>	<i>i Wx</i>	<i>I Wx</i>	2	11452
12	<i>c wx</i>	<i>C Wx</i>	<i>c Wx</i>	<i>C Wx</i>	3	Emerson 1921, table 1
Total					6	
13	<i>y bh</i>	<i>Y Bh</i>	<i>y bh</i>	<i>Y Bh</i>	10	11458, 13553

Of a total of 127 mosaic seeds (items 1-12 of Table I) involving the aleurone factors *C c* or *I i*, together with one or both of the endosperm factors *Wx wx* and *Sh sh*, known by genetic tests to form a linkage group, 121 seeds (item 1-9) showed an exact correspondence in the aberrant part between the recessive aleurone color and the underlying recessive endosperm character of the female parent, the normal part of the seeds showing in all cases the dominant aleurone and endosperm characters of the male parent. In case of one seed (item 10) the spot of recessive aleurone color was underlaid in part by waxy and in part by corneous endosperm. That is, the correspondence between the recessive aleurone color and the underlying recessive endosperm texture was not exact. Of the remaining five mosaic seeds (items 11, 12) the aberrant spot of recessive aleurone color was underlaid by the dominant endosperm texture. Thus, in the great majority of cases the aleurone and endosperm characters of the *C* linkage group are associated in mosaic seeds.

Similar evidence of the relation of linked characters in mosaic seeds is available for only one other group, namely, that involving the linked genes *Y y* for yellow and white endosperm and *Bh bh* for blotched aleurone-color pattern. In all the ten mosaic seeds observed (item 13 of Table I) the yellow part of the endosperm was overlaid by blotched aleurone and the white part of the endosperm was overlaid by colorless aleurone. There is, therefore, sufficient evidence to indicate that mosaic seeds involving the *Y* linkage group are due to some aberrant chromosome behavior such as non-disjunction or elimination, just as are the great majority of those involving the *C* group.

If the results reported are representative of other chromosomes and if the conclusions drawn from them are sound, it would seem justifiable to use evidence from aberrant endosperm development to determine whether other aleurone and endosperm genes are linked or independently inherited, in short, to determine whether their *loci* are in the same or in different (non-homologous) chromosomes.

Mosaic seeds from crosses involving various aleurone and endosperm characters not known to be linked are listed in Table II.

That the *C* linkage group is distinct from the *Su* group is indicated by the data from 41 mosaic seeds (items 1-7 of Table II). In 14 of these seeds (items 3, 4) the aberrant part had the recessive sugary endosperm, but the overlying aleurone showed the dominant aleurone color, *C*, like that of the normal part. The aberrant part of the other 27 seeds exhibited characters due to one or more of the recessive genes *c*, *sh*, *wx*, but had the dominant starchy, *Su*, endosperm throughout. The evidence that the factor pair *Su su* belongs to a different chromosome pair from the one carrying *C c*, *Sh sh* and *Wx wx* is particularly strong when items 6 and 7 are considered. Here the linked recessives, in one case *c* and *wx* and in the other *c*, *sh* and *wx* were definitely associated in the aberrant spot, thus showing clearly some chromosome aberration, but the other recessive characters of the female parent due to the genes *su* and *y* were not developed.

That the *C* linkage group is distinct from the *Y* group is similarly shown by the 7 mosaic seeds (items 6-11 of Table II) involving the endosperm-color pair *Y y* and one or more of the aleurone and endosperm pairs *C c*, *Sh sh* and *Wx wx* of the *C* group. That the *C* and *R* linkage groups are distinct is indicated by 16 mosaic seeds (items 12-14). The independence of

TABLE II
ABERRANT SEEDS OF MAIZE INVOLVING NON-LINKED GENES

Item No.	Genotypes of parents		Phenotypes of mosaic seeds		Number of Mosaic seeds	Citation of published data, and pedigree number for new data
	Female	Male	Aberrant part	Normal part		
1	c su	C Su	c Su	C Su	20	Emerson 1921, table 3
2	c su	C Su	c Su	C Su	2	11458
3	c su	C Su	C su	C Su	5	Emerson 1921, table 3
4	c su	C Su	C su	C Su	9	11453, 11454
5	c su y	C Su Y	C su Y	C Su Y	3	11458
6	c wx su y	C Wx Su Y	c wx Su Y	C Wx Su Y	1	12293
7	c sh wx su y	C Sh Wx Su Y	c sh wx Su Y	Sh Wx Su Y	1	12293
8	c y	C Y	c Y	C Y	2	Emerson 1921, p. 418
9	c y	C Y	c Y	C Y	1	13721
10	c wx y	C Wx Y	c wx Y	C Wx Y	1	11454
11	c sh wx y	C Sh Wx Y	c sh wx Y	C Sh Wx Y	1	11454
12	wx su y r	Wx Su Y R	Wx Su Y r	Wx Su Y R	9	11461
13	wx su r	Wx Su R	Wx Su r	Wx Su R	1	11461
14	wx r	Wx R	Wx r	Wx R	6	Emerson 1921, table 3
15	wx a	Wx A	Wx a	Wx A	12	Emerson 1921, table 3
16	wx pr	Wx Pr	Wx pr	Wx Pr	20	Emerson 1921, table 3
17	wx pr	Wx Pr	Wx pr	Wx Pr	2	11452
18	c wx pr	C Wx Pr	C wx pr	C Wx Pr	1	10171
19	c pr	C Pr	C pr	C Pr	4	10345, 12445, 13721
20	su y	Su Y	su Y	Su Y	8	11458, 11460
21	su y	Su Y	Su y	Su Y	1	11458
22	su y bh	Su Y Bh	su Y Bh	Su Y Bh	4	12287, 13553
23	su y r	Su Y R	su Y R	Su Y R	6	11459, 11460, 11696
24	su y r	Su Y R	Su Y r	Su Y R	28	11460, 11461
25	su r	Su R	su R	Su R	6	Emerson 1921, table 3
26	su r	Su R	su R	Su R	5	11459
27	su r	Su R	Su r	Su R	13	Emerson 1921, table 3
28	su r	Su R	Su r	Su R	9	11459, 12450
29	su y a	Su Y A	Su Y a	Su Y A	4	11464
30	su y a	Su Y A	su Y A	Su Y A	1	13719
31	su a	Su A	su A	Su A	2	11464
32	su pr	Su Pr	su Pr	Su Pr	2	Emerson 1921, table 3
33	su pr	Su Pr	Su pr	Su Pr	1	Emerson 1921, table 3
34	su pr	Su Pr	Su pr	Su Pr	1	12289
35	su r pr	Su R Pr	Su R pr	Su R Pr	1	12450
36	y a	Y A	Y a	Y A	16	11465, 11466, 12290, 13719
37	y pr	Y Pr	Y pr	Y Pr	2	11466
38	a pr	A Pr	A pr	A Pr	9	11690, 11691, 13720, F207
Total					220	

the *C* and *A* groups is shown by the 12 mosaic seeds (item 15) involving *Wx wx* and *A a*. The 27 mosaic seeds involving the aleurone-color pair *Pr pr* and one or both of the pairs *C c* and *Wx wx* (items 16-19) indicate that the aleurone pair *Pr pr* does not belong with the *C* group.

The independence of the *Su* and *Y* groups is shown by 23

mosaic seeds (items 5, 20–23, 30, of Table II) all involving the factor pair *Su su* and one or both of the pairs *Y y* and *Bh bh* of the *Y* group. The 77 mosaic seeds involving *Su su* and *R r* (items 12, 13, 23–28) show independent inheritance between the *Su* and the *R* groups. The evidence that the *Su* and the *A* groups are distinct is limited to 7 mosaic seeds (items 29–31). The 5 mosaic seeds (items 32–35) available to show the relation of the pairs *Pr pr* to the *Su* group indicate independence.

That the *Y* and *R* groups are distinct is shown by 37 mosaic seeds (items 12, 24, of Table II). The independence of the *Y* and the *A* groups is indicated by 20 seeds (items 29, 36). Only two mosaic seeds (item 37) have been found to involve *Y y* and *Pr pr*. These two give no indications that the pair *Pr pr* belongs to the *Y* group.

That *Pr pr* does not belong to the *A* group is indicated by 9 mosaic seeds (item 38 of Table II). Only one mosaic seed (item 35) is available as a test of the relation of *Pr pr* to the *R* group and this gives no indication of linkage. If *Pr* were linked with *R r* or with *A a*, no direct evidence of the fact could be obtained from mosaic seeds. When either recessive *r* or recessive *a* is homozygous, neither purple nor red aleurone color develops, and it is therefore impossible to determine whether *Pr* or *pr* is present in an aberrant spot.

For the same reason it is impossible to obtain evidence of the relation of *R r* to *A a* by the mosaic-seed method. Obviously, when *r* is homozygous, the lack of aleurone color thus assured makes it impossible to determine whether *A* or *a* is present. And, similarly, homozygous *a* makes it impossible to determine the presence of *R* or *r*. It will be possible to test the relation of *R r* to *A a* only when, if ever, there is discovered some other endosperm factor linked with one or the other of these factor pairs, as *Sh sh* and *Wx wx* are with the aleurone pairs *C c* and *I i*.

By way of summary, it is to be noted that there have been made all the comparisons that are possible at present, by means of the aberrant-endosperm method, to determine the linkage relations of the several aleurone and endosperm factor pairs of maize. True, it can not be claimed that wholly adequate data have been obtained for all these comparisons. It may fairly be concluded, however, that the 137 mosaic seeds recorded in Table I afford strong evidence that, with respect to the characters belonging to the *C* and to the *Y* linkage groups, mosaic seeds are due to some

aberrant chromosome behavior such as non-disjunction or elimination. If mosaic seeds involving other endosperm characters are due to the same behavior of other chromosomes, the data reported in Table II for 220 mosaic seeds may be used in an attempt to determine whether certain linkage groups are actually distinct. The extent of these data is shown in Table III.

TABLE III

SUMMARY OF THE NUMBERS OF MOSAIC SEEDS OBSERVED IN TESTS INDICATING INDEPENDENT INHERITANCE OF CERTAIN LINKAGE GROUPS IN MAIZE

Linkage Groups	<i>Su</i>	<i>Y</i>	<i>R</i>	<i>A</i>	<i>Pr</i>
<i>C</i>	41	7	16	12	27
<i>Su</i>		23	77	7	5
<i>Y</i>			37	20	2
<i>R</i>				0*	1
<i>A</i>					9

Except for the relation of the *R* group to the *A* group, which can not be tested by the mosaic-seed method, the evidence in most cases is sufficiently extensive to indicate that the *C*, *Su*, *Y*, *R* and *A* linkage groups are distinct. The evidence is fairly good also that the *Pr pr* factor pair does not belong to either the *C*, *Su* or *A* groups, and there is some evidence that it does not belong to either the *Y* or the *R* group. It is noteworthy that in no instance was there observed a single mosaic seed that suggested linkage between any of the groups here under consideration.

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* No tests are possible (see text).

SHORTER ARTICLES AND DISCUSSION

THE VARIABILITY IN WEIGHT OF LEGHORN CHICK- ENS AT HATCHING, THIRTY-FIVE DAYS AND MATURITY¹

THERE have been many studies upon the growth of the chicken and yet none, so far as I am aware, have given any information concerning the variability of the chick at different ages, nor the correlation between the weights at different periods of growth. This little study which is based upon nearly 2,000 weighings is an attempt to give some information upon these two questions.

The chickens used were single-comb White Leghorns which were hatched and raised by the department of poultry husbandry of the University of Nebraska. The weighings were made in connection with another problem, but these weights were turned over to me by Professor Frank E. Musschl and I wish to thank him most heartily for his kindness in placing these data at my disposal. I wish to thank Miss Lois Pedersen who helped in making some of the computations.

These chickens were artificially hatched and reared. They were hatched at various times between February 12 and May 11, 1922. Each chick was marked by a wing band and later by a leg band, so the weight of each individual chick is available for each period.

There were 510 males and 452 females weighed at time of hatching, 414 males and 379 females of the above chicks were weighed when they were 35 days old and 239 of the females were weighed when they began to lay, thus making a total of 1,994 weighings. The age at the time the first egg was laid was determined for 263 of these pullets. The pullets were trapnested and the weight of 239 of the 263 was obtained before they were released. The weighings at the time of hatching and at 35 days were accurate to one gram and the weighings at the beginning of laying were made in pounds and accurate to a quarter of a pound. These weights have been changed to grams to correspond to the other weights.

¹ Studies from the Zoological and Anatomical Laboratories of the University of Nebraska, No. 138.

The average for the weights of the males and females at hatching and at 35 days and for the females at time of sexual maturity and also the average age of sexual maturity is shown in the first line of Table I. The second line shows the range, the third the standard deviation and its probable error and the fourth line shows the coefficient of variability and its probable error for each weight and the age (last column) at the time the first egg was laid. The first two columns show the hatching weight, in grams, of the 510 males and the 452 females, the next two columns show the weight at 35 days of the 414 males and the 379 females, the fifth column shows the weight in grams of the 239 females at the time they began laying. The last column shows the age at which the 263 females began laying. The formulae used in determining the standard deviation, the coefficient of variability and the coefficient of correlation and the probable error of each are those given by Davenport.²

The data upon the growth of the Leghorn chicken given by Buckner and associates,³ and Card and Kirkpatrick⁴ have been plotted in connection with similar data collected by Latimer,⁵ and the averages for the weights of these chicks at the three periods when plotted on the same chart are found to be very close to the other curves for the first two periods and are slightly heavier than the chickens reported by Latimer at 210 days. Buckner and associates carried their study only to the end of the 24th week and Card and Kirkpatrick carried their experiment to the end of the 28th week.

The coefficients of variability which are the best measures of the amount of variation are shown in the last line of Table I. These show that the chickens are the most uniform in weight at time of hatching, and the least uniform at 35 days. The coefficient of variability for the females at hatching being 8.09 ± 0.18 and at 35 days of age, 21.78 ± 0.56 , or 2.69 times the coefficient at time of hatching. The coefficient of variability for the weight at the beginning of laying is 11.81 ± 5.97 or 1.46 times the coefficient of the hatching weight of the females. Thus, these chickens show the maximum variability in weight at 35 days, less variability at sexual maturity and the minimum, at time of hatching.

² "Statistical Methods," John Wiley and Sons (1904).

³ *Am. Jour. Physiol.*, Vol. 47, pp. 393-398 (1918).

⁴ *Bull. Storrs Exp. Sta.* No. 96 (1918).

⁵ Unpublished Material. Abstract, Vol 2, Papers from the Mayo Foundation and the Medical School, University of Minnesota.

The chick at 35 days has passed its initial period of slow growth, including the first three or four days during which, like the human, its weight is less than at the time of hatching, and by this time it has begun to grow rapidly. Just before sexual maturity, as shown by the beginning of laying in the pullets, the growth curve flattens out, indicating a retardation of the growth rate. Thus, at hatching and at sexual maturity the growth rate is less rapid and the variability in weight is less than at 35 days of age when the rate of growth is very rapid. This shows that in chickens as well as other animals, in which this has been studied, a period of rapid growth is a period of greater variability. Unfortunately, the available data give no means of determining just when this maximum variability occurs in the growth of the chicken.

Jackson⁶ in his study of the variability in the weight of the albino rat reports the lowest coefficient of variability (12.3 ± 0.64) at birth. This rises to a maximum of 28.4 ± 2.1 at 20 days of age and then drops to 19.1 ± 1.5 for 5 months of age. These coefficients of variability are for both sexes combined. Porter⁷ in his study of St. Louis school children finds the maximum variability in weight of girls at 13 and 14 years and at 16, for the boys.

The coefficients of variability for both the males and females at hatching and at 35 days are shown separately in Table I. The coefficient of variability is shown for the females only at time of the first egg. The females are slightly more variable at hatching, the coefficient of variability being 1.097 times as large as that of the males. At 35 days of age this condition is reversed and the males have a coefficient of variability 1.05 times greater than that of the females.

Jackson finds the coefficient of variability for the male albino rats, at all ages, greater than that for the females except at 6 weeks, when the females have a coefficient of variability 1.4 times as great as that for the males. Hatai⁸ finds the coefficient of variability of the adult male albino rat about 2.1 times that for the female, or 25.076 ± 2.675 and 12.235 ± 0.974 , respectively. Porter finds a greater variability in the weight of the girls from 6 to 13 years inclusive, and from 14 to 17 years inclusive the boys show the greater variability.

⁶ *Am. Journal Anat.*, Vol. 15, pp. 1-68 (1913).

⁷ *Trans. St. Louis Acad. Sci.*, Vol. 6, pp. 233-250 and 263-426 (1894).

⁸ *Am. Jour. Anat.*, Vol. 7, pp. 423-441 (1908).

TABLE I

	Hatching weight		35-day weight		Mature weight females (239)	Age (days) mature females (263)
	males (510)	females (452)	males (414)	females (379)		
Average	39.45 ± 0.09	39.16 ± 0.10	209.15 ± 1.60	191.71 ± 1.45	1626.51 ± 8.38	209.81 ± 1.09
Range	32-50	30-48	66-351	83-297	1140-2268	153-303
Standard deviation	2.91 ± 0.06	3.17 ± 0.07	48.39 ± 1.13	41.75 ± 1.02	192.14 ± 5.93	26.27 ± 0.77
Coef. of variability	7.37 ± 0.16	8.09 ± 0.18	23.14 ± 0.54	21.78 ± 0.56	11.81 ± 5.97	12.52 ± 0.78

TABLE II

Coefficients of correlation	
Hatching weight and 35-day weight—Males	+ 0.2202 ± 0.0259
Hatching weight and 35-day weight—Females	+ 0.1042 ± 0.0344
Weight at 35 days and maturity—Females	+ 0.0061 ± 0.0436
Weight at 35 days and age at maturity—Females	— 0.2672 ± 0.0407

Correlations: The data at hand give the weight of the individual chickens at two periods for the males, namely, at hatching and at 35 days, and for three periods for the females, at hatching, 35 days and at the beginning of laying and so these data have been used to find out to what extent larger chicks at hatching are large chicks at 35 days and also what relationship there is between the weight of a 35-day-old female chick and the same chicken at the beginning of the laying period. A statement frequently made by poultrymen is that the best laying pullets begin to lay at an early age and so an attempt has been made to determine the relationship between weight at 35 days and the age at which the pullet began to lay.

Table II shows the coefficients of correlation between hatching weight and weight at 35 days for both the males and females. The third correlation is between the weights at 35 days and at the time of sexual maturity, and the last correlation is between the 35-day weight of the females and the age at the time the first egg was laid.

The first two correlations ($+0.22$ and $+0.10$) are so low that they are not significant. In other words, there is very little probability that a large chick, at hatching, either male or female, will grow into a large chick at 35 days. Similarly, there is practically no correlation between the 35-day weight and the weight at time of sexual maturity. This correlation is but $+0.006$ and its probable error is ± 0.044 , or over seven times the correlation, so this may be considered as of no significance. The correlation between weight at 35 days and age at which the pullet began laying shows a slightly higher correlation, although it is a negative correlation. This correlation is the highest numerically of any of the correlations, and so this would indicate that there is a greater probability of a large female chick at 35 days beginning to lay earlier than the average. This is, however, not high enough to be called a significant correlation. The commercial poultryman would be delighted if he could pick the most productive pullets at an early age and market the others together with the surplus cockerels at the end of the maximum growth period, but the above correlations do not indicate a sure method for making such a selection. A correlation between the body weight a little later on and the age of sexual maturity might give a more significant correlation, but the data at hand does not justify any predictions of the size or age of sexual maturity based on the weight at 35 days.

SELECTIVE ACTION OF STRYCHNIN AND NICOTIN ON
A SINGLE CELL¹

THE greater amount of work done on the action of drugs on unicellular forms concerns the problem of efficiency of antiseptic and disinfectant drugs of various concentrations. There is a relatively small number of investigations of other types. The observations to be reported in the present paper were obtained by applying strychnin and nicotin in different concentrations to some of the smaller ciliates which developed in a hay infusion prepared in the laboratory. The form to which special attention was given is *chilodon megalotrocha*, Stokes. (See Figures 1 and 2).

The results show that strychnin and nicotin act selectively on certain portions of the one-celled animal. This can be shown by applying the drug in varying concentrations. It is difficult to understand this selective action on the single cell unless we consider that, as Rees² has demonstrated, the infusorian possesses structures which are comparable to ordinary neuro-muscular mechanisms. Rees has shown clearly that the paramecium possesses a neuro-motor apparatus. The nervous elements at the periphery consist of fine branching fibrils. These converge to a neuro-motor center. At the periphery these are connected with the basal granules of the cilia. If we accept the statements made by some investigators that the cilia are the ends of myoid fibrils and that the physiological behavior of these elements is similar to that of the fibrils of skeletal muscle, we may say that the selective action of drugs involves the idea of mutual antagonism or inhibition among the fibrils of the different portions of the body of the ciliate.

I find it profitable to consider that the neuro-motor center inhibits or correlates the action of a number of more or less independent functional units of the cell, and that this correlation is responsible for the typical movements of the animal in swimming—this is a forward movement with a greater tendency to turn to the left than to the right. There is also a tendency of the animal to turn over and over on its long axis as it swims, but this occurs very seldom under normal conditions. The functional units of the animal differ chemically among themselves,

¹ From the Department of Physiology and Pharmacology, Marquette University Medical School, Milwaukee, Wisconsin.

² Rees, C. W., AMERICAN NATURALIST, 1921, 55, 464-469.

the criterion being the selective action of certain drugs. Certain drugs affect some parts of the cell more than others, and this results in the special forms of reaction of the animal which are to be reported.

When a drop of the water containing the animals is treated with either a solution of strychnin or nicotin, the animals generally assemble and remain in small groups. Presumably, this is due to a greater chemical affinity existing between them after the drug is applied. A special factor responsible for the perceptible reactions attributed here to the increase in chemical affinity is the motility of the animals. If the individuals tend to collect anyway, because of the addition of the drug, any movement of the cilia will only hasten the process.

After this grouping occurs, the animals begin to respond in one specific way—those treated with a drop of 1: 1000 solution of strychnin sulphate rotate in the counter-clockwise direction with the posterior in advance of the anterior end. It is best to apply the solution along one side of the coverglass and allow it to become distributed gradually throughout the water. The oral groove is to the right of the animal as long as the turning in this direction lasts. Figure 1 represents the position of the animal during this time. The curved arrow, S, indicates the direction of movement due to strychnin. A indicates the anterior and P the posterior end of the animal. Figure 1 represents the animal in its normal position; it is here resting on its ventral surface. However, a specimen is occasionally found resting on its dorsal surface, as is shown in Figure 2.

From the time that the 1: 1000 solution of strychnin sulphate is applied until the animals die, the amount of activity becomes gradually less. When the turning first begins the anterior end of the body is almost stationary, if not entirely so, but after a few minutes of turning each animal describes large circles by moving very slowly, posterior end first, in the counter-clockwise direction. The change from the small to the relatively large circles is a gradual process. We may profitably assume that the drug affects certain portions of the cell more than others. The circular or spiral movement would be due, then, to the selective action of the drug upon the organism. The strychnin depresses all the cilia, affecting those on the right more than those on the left. It is also true that the anterior cilia are depressed, but less than those on the right or left. The posterior cilia are depressed

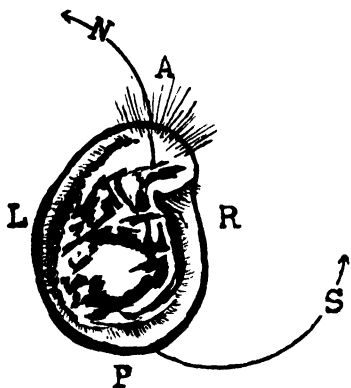


FIG. 1

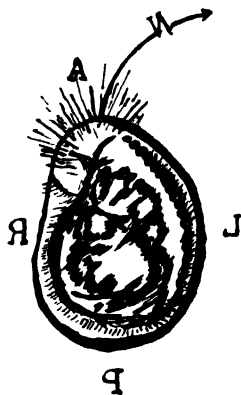


FIG. 2

FIG. 1. Animal resting on ventral surface. L, left side; R, right side; A, anterior end; P, posterior end; S, direction of movement due to strychnin; N, direction of movement due to nicotine.

FIG. 2. Animal resting on dorsal surface, which is one stage of nicotine poisoning. The letters (inverted) have the same meanings as in Fig. 1.

least of all. It could not be observed that strychnin increased the action of any of the cilia; depressing effects only were observed. The rotation in the counter-clockwise direction with the posterior end first is due to the relatively great activity of the posterior cilia which execute their rapid or driving strokes from left to right. The strychnin either decreases the action or leaves functionless the cilia of the right side, left side and anterior end; and since those at the posterior end are still relatively very active these necessarily turn the animal in the direction indicated by the curved arrow S. As the cilia on the right side, left side and anterior end become less and less active the animal ceases gradually to make the small sharp turns resulting in very small circles and makes the larger ones which cease only when the cilia at the posterior end also become inactive.

When a 1: 1000 solution of nicotine is applied along the sides of the coverglass and allowed to become distributed gradually throughout the water, the animals first form groups and then each of them turns in the counter-clockwise direction similarly as when strychnin is applied. In this instance, however, the animal moves forward instead of backward. The direction of movement is indicated by the curved arrow N in Figure 1. This difference is due to the fact that in nicotine poisoning the posterior cilia are depressed sooner than the anterior cilia. These anterior cilia make their rapid or driving strokes from left to right, which

results not only in pulling the cell forward but also in continually forcing the anterior end to the left. After a time, however, a certain irregularity in the movements of each of the animals occurs, but this does not mean an increase in the activity. The animal now swims forward, turning on its long axis as it goes. Finally it comes to a standstill, but it is now resting on its dorsal surface. The oral groove is on the left instead of on the right side of the animal. Figure 2 shows this abnormal position. The cilia seem to be functioning too weakly to cause any movement of the body as a whole. In a few seconds, however, the animal begins to turn in the clockwise direction, the posterior end being almost if not entirely stationary. Small circular movements of the entire body develop, and these become gradually larger, until the anterior cilia cease beating and the animal dies.

The turning on the long axis as the animal swims is due to the persistent action of the cilia near the mouth of the oral groove, after other cilia in the groove and elsewhere on the body have stopped beating. It can be observed easily that these large cilia are the last of those in the groove to stop beating, and that when the animal is resting on its ventral surface these cilia execute their rapid or driving strokes in the downward and slightly backward direction. This causes the animal to rotate on its long axis and to move at the same time in the counter-clockwise direction. As soon as these cilia weaken or cease beating entirely the animal comes to rest, generally on its dorsal surface. It is after this that the anterior cilia, which are still active, drive the animal in a spiral fashion in the clockwise direction when viewed from the ventral surface or in the counter-clockwise direction when viewed from the dorsal surface. When the animals are observed from their ventral surfaces, the spiral movement is in the clockwise direction with respect to the position of the observer. However, if we imagine ourselves observing the inverted animals from their dorsal surfaces, *i.e.*, from underneath the microscope, they would be turning in the counter-clockwise direction as usual.

The turning on the long axis does not occur in strychnin poisoning because the cilia of the groove are among the first to be paralyzed. This is, therefore, only another special observation concerning the selective action of drugs on the single-celled animal.

SUMMARY

(1) The animals show a tendency to collect in small groups soon after strychnin or nicotin is applied.

(2) Strychnin acts specifically on the body of the animal, selecting and depressing especially the locomotor organs on the right and at the anterior end of the cell. The posterior cilia and the still active cilia on the left then turn the animal in the counter-clockwise direction, with the posterior in advance of the anterior end. Eventually, all the cilia are paralyzed. The animals remain on their ventral surfaces.

(3) Nicotin also acts selectively on the body of the animal, depressing especially the posterior cilia and those on the left side of the cell. The only cilia on the right which are not depressed early are the long ones at the mouth of the oral groove. As long as an animal remains on its ventral surface, these cilia of the groove and the still active ones at the extreme anterior end turn the cell in the counter-clockwise direction with the anterior in advance of the posterior end.

(4) Some time after nicotin is applied the animals cease turning in the counter-clockwise direction, respond irregularly for a short time, and eventually go forward turning over and over as they go. This turning of an animal on its long axis is due to the action of the cilia at the mouth of the groove which normally tend to cause the organism to rotate in this way, but which ordinarily fail to cause the rotation because other cilia (which are now depressed) overcome the effects of the groove cilia and keep the animal on its ventral surface. It is only after certain cilia, presumably those on the left side, are paralyzed that the cilia of the groove can turn the animal as they normally tend to do. The turning over and over as the poisoned animals go forward ceases finally, as the cilia of the groove become depressed, and then the great majority of the animals come to rest on their dorsal surfaces. After this the animals turn again in the spiral fashion in the counter-clockwise direction with respect to their dorsal surfaces. All the cilia are eventually paralyzed.

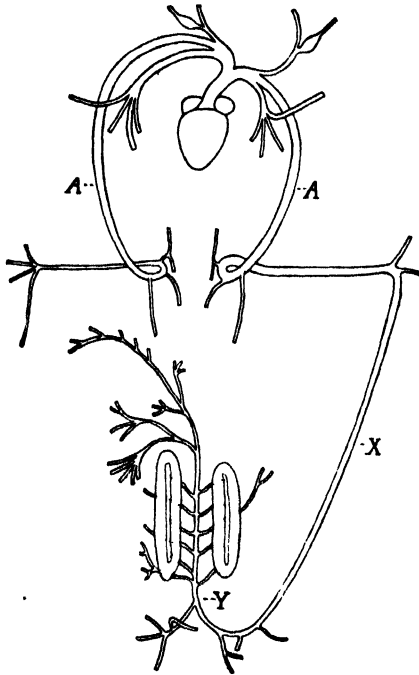
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ABNORMAL CIRCULATORY SYSTEM OF A FROG

RECENTLY a frog (*Rana pipiens*) was found with such an abnormal circulatory system that it seems worthy of record. In so far as I was able to discover the venous system was normal in all respects.

The accompanying diagram shows the arrangement of the main arterial trunks. The chief difference between this individual and the normal frog is that the systemic arches (A, A) never unite to form a dorsal aorta. The blood for the abdominal viscera and the posterior part of the body is supplied instead by a large trunk (X) from the subclavian. This trunk joins at the posterior end with a vessel (Y) which is the same as the dorsal aorta in its position and which supplies the same parts of the body as the latter. It differs from it, however, in that it is not formed by the fusion of the systemic arches and that in it the blood flows in an anterior instead of a posterior direction as is usual in the aorta. The liver has no arterial blood supply. The systemic arches



(A, A) are present and near their origin appear normal but neither extends posterior to the subclavian except as small, rudimentary vessels which never join but lie beside the vertebral column. The vessel X which replaces the aorta is not in the abdominal cavity but passes outside the peritoneum and lies dorsal to the spinal nerves.

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THE ARTIFICIAL INDUCTION OF SYMMETRICAL CLAWS IN MALE FIDDLER CRABS

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THE observations and experiments of a year ago seemed to show beyond much doubt that the young male fiddler crabs pass through a stage in which two large claws are present. The loss of one or the other of these claws is the determining factor leading to the fixed asymmetry of the later stages. The claw that is left becomes the "fiddle," the new claw that replaces the lost one becomes the permanent small claw. The number of cases on which these conclusions rested was not large and the possibility that the induced change was only temporary was not entirely excluded. Therefore I have gone over the same ground again with larger numbers. The results are here recorded. They confirm and extend the earlier findings. *A new and significant result was obtained by removing simultaneously both of the large claws of the young males. Two small claws were regenerated and both remained small throughout later molts.* In other words, if, in the critical stages, the male crab does not become unbalanced by the loss of one of its claws, it loses the power to develop the normal asymmetry.¹

YEARLING CRABS WITH ONE CLAW ABSENT WHEN COLLECTED

The first young crabs were collected on the beach at West Falmouth on June 12, 1923. These had come ashore

¹ The records and the measurements of the crabs were made by Mrs. A. H. Sturtevant to whose skill and accuracy I am greatly indebted.

in the preceding summer, in July or August, or perhaps later. These were the smallest crabs to be found at this time (June 12). Many of them had one large and one small claw, but there were also 106 male crabs that had lost one claw. Exactly half of them (A) had lost the right claw and half (B) the left one. The former (A) were kept in glass dishes and fed until the next molt when the missing (left) claw reappeared. One escaped, 4 died. Forty were killed after the first molt. The new (left) claw was a small one in all cases. In order to meet the possible objection that these left claws were small because they had had only the time before the next molt to regenerate (an objection of no weight as other results have shown) eight of these crabs were kept alive until the next molt. The left claw remained small (like that of the female) and the right claw remained large. Both showed the normal increase in size that occurs at each molt.

The other half (53) of the collected crabs that had lost the right claw and had a larger left claw were also kept until the next molt (except two that died). Forty-four were killed after the molt. In all, the new right claw was small. Seven were kept until another molt had taken place. The right claw was still small and the left large. One of the seven had, however, dropped its larger claw after the first molt (11 days before the second molt), but this did not affect the outcome since the right claw was small and the left one large. In other words the "set" had taken place.

The preceding results harmonized with previous ones. All these male crabs had presumably reached the stage when two large claws were present and during the winter or spring had lost one of them. Had they remained on the beach they would all have become right or left handed at the next molt as they did in the laboratory.

ISOLATION OF DOUBLE CLAWED MALES

In order to find out how the double clawed young males lost one of their claws and also to find out what

happens in case they do not lose a claw or lose both, fifty-one were isolated, each in a small petri dish with a little sand and water. They were fed every day or two with small pieces of meat (clam). Seventeen died before molting, four escaped; the remainder went through one or more molts and both claws remained large, thus:

7	passed	through	one	molt	then	died
8	"	"	two	molts	"	"
4	"	"	three	"	"	"
2	"	"	four	"	"	"

There were in addition 9 males (of the 51) that dropped one claw. One of these died before molting. Seven dropped a left claw and after the next molt they were found to have regenerated in its place a small left claw. One of these (7) had its large right claw removed while still soft. It replaced this at the next molt by a large right claw. This claw was removed a second time and a large one again came back. The asymmetry had become fixed. Three crabs (of the seven) died before a second molt, three lived through a third molt and two lived to molt once more. No further change in the asymmetry took place.

Of the nine isolated males, one lost the right claw and regenerated a small right claw. From this one the large left claw was removed while it was still soft (after the molt). It regenerated a left large claw at the next molt in place of the large one removed.

REMOVAL OF ONE CLAW OF DOUBLE CLAWED MALES

From 21 small crabs with two large male claws either the right (A) or the left (B) claw was removed. In ten of these (A) in which the right claw was removed five died before regeneration, five regenerated small claws on the right side. From one of the latter the large left claw was removed eight days after molting. It regenerated a large left claw at the next molt in place of the large one removed. Two of the five crabs were kept alive through another molt. No further change occurred.

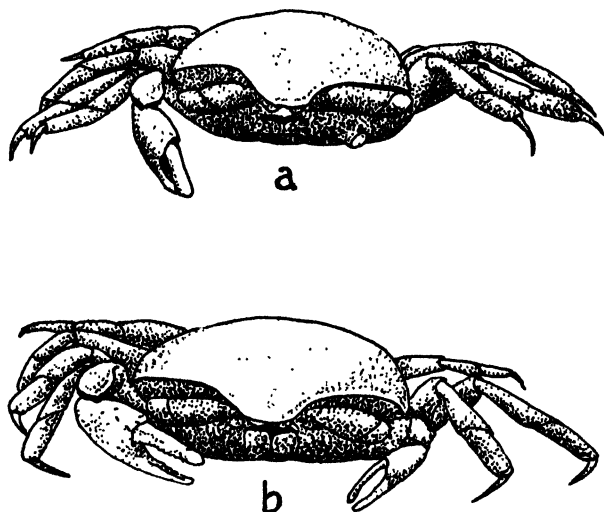


FIG. 1. The upper figure (a) is from a male (four millimeters) with two large claws like the one in the drawing. The left claw was removed. The lower figure (b) represents the same crab after the next molt (four and a half millimeters) when the left claw was replaced by a small claw.

In eleven double clawed crabs (B) the left claw was removed, six died before molting, four regenerated a small left claw. From one of them the large right claw was removed several days after the first molt. A large right claw regenerated in place of the one removed. The asymmetry was fixed.

THE LOSS OF ONE LARGE CLAW FROM OLDER DOUBLE CLAWED MALES

The smallest males with two large claws measure about two and a half millimeters across the thorax. At each successive molt there is a gain of about one millimeter. Amongst the crabs collected with two large claws were some that measured three to four millimeters. The largest isolated males, with two big claws, measured five and a half millimeters. Presumably most of the crabs that were collected above three millimeters and which had equal claws had also acquired them earlier and retained them through more than one molt. The isolated crabs with two large claws molted once or twice before losing a claw. One of these double clawed males lost its left claw

after it had molted and then replaced the one lost by a small claw; five others with big claws molted twice and then lost one regenerating a small one in its place at the next molt (four lefts, one right). It is evident that the male may retain its two large claws for at least two molts and still regenerate a small one if one of them is lost.

REMOVAL OF BOTH CLAWS FROM DOUBLE CLAWED MALES

From twenty double clawed males both claws were removed. One died, 19 regenerated both claws which were small. Of these three died before the next molt, nine molted again and both claws remained small, one molted another time and both claws remained small and one molted a third time and remained as before.

These results show that if both large claws are simultaneously removed from double clawed males, they are both replaced by small claws. That the crabs can not subsequently become asymmetrical is shown by later molts. Of course, it is possible that with large numbers, cases might be found when asymmetry appears, but the number suffices, I think, to show that if at this critical stage the symmetry is preserved, as here, by removing both big claws, the crab has lost the opportunity to develop the normal asymmetry of the species.

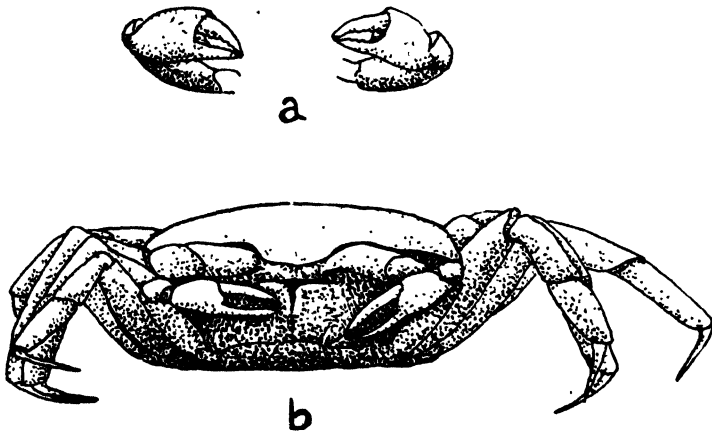


FIG. 2. The upper figure (a) represents the pair of "large" claws removed from a male crab (four millimeters). The lower figure (b) is the same crab after the next molt when two small claws replace the two large ones.

The experiment was carried further as follows. After the two small claws (replacing the two large ones) had appeared, one of them, the right, was removed from three crabs and one of them, the left, from two other crabs. In all cases the results were the same, a small claw appeared after the next molt in place of the small claw removed, and the opposite small claw remained small. In other words, it was no longer possible to produce asymmetry by removing one of the two small claws. Their character had become fixed.²

EVIDENCE THAT THE MALE DEVELOPS TWO LARGE CLAWS
BEFORE IT DEVELOPS A LARGE AND A SMALL CLAW

In the several collections that were made, especially during the later summer, many very small crabs were collected, that as a rule were smaller than the double clawed male stage. Many of these were kept alive until they molted. Thus 192 crabs (out of 295) remained alive until the next molt. Of these 43 became double clawed males. Since half of these crabs should be females, and since some of them had not, presumably, reached the double clawed stage at the next molt, the appearance of so many (43) double clawed males is a clear indication that most probably all males pass through this stage unless at the preceding stage one of the claws has been already lost. That such a loss may lead to the remaining claw becoming the large one had been shown by my experiments of last year. The number of crabs in those experiments was small. Therefore I repeated the experiment again with the following results. From 44 small crabs with equal small claws the left claw was removed. Twenty-one died before molting; 11 regenerated two small claws, 4 regenerated a right large claw. Of these one died after the molt, one after another molt, one after a third molt and one after a fourth molt. The asymmetry remained in all cases. The results are the same as those

² There is a possibility that in both cases the original small claw was removed.

of last year and indicate that the asymmetry may also be induced if at the stage just before the double clawed male stage one claw is removed.

CONCLUSION

These and the preceding experiments show that the asymmetry of the male fiddler is induced in the young crabs by the accidental loss of one (right or left) claw. If both large claws of the young male stage in which two large claws are present are simultaneously removed no large claws develop but the male remains with two small claws through later molts. The other sexual characters—the abdominal appendages—develop in these small clawed males in the same way as in asymmetrical males. The double clawed males may retain their double claws throughout several later molts, but still retain the power to become asymmetrical if one of the claws is lost. Whether there comes a still later stage when the replacement of a large by a small claw is no longer possible has not yet been shown.

SPECIES HYBRIDS IN CREPIS AND THEIR BEARING ON EVOLUTION¹

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To ascertain and evaluate the natural processes by which the thousands of species of animals and plants, both living and extinct, developed from preexisting species is one of the most interesting and difficult of biological problems. Indeed, there are eminent scholars who assert that the world is little nearer the solution of this great problem than it was a century ago. On the other hand, there are some who claim to have found the solution in a single natural process or method and that it and it alone will solve the whole riddle. However, in view of the evidence at our command, neither of these attitudes is justifiable and, while it may remain for generations yet unborn to comprehend fully and in their true relations the various evolutionary processes, yet there can be no doubt that the utilization of the experimental method of attack is bringing us nearer to a point of vantage from which we can at least survey the realm of nature with more certainty as to interpretation and map out campaigns that will lead ultimately to the desired solution.

Time forbids even a brief discussion of the evolutionary theories generally associated with the names of Lamarck and Darwin. Since Guyer's well-known experiments on the development of antibodies in rabbits that had been injected with serum from fowls inoculated with rabbit eye lens, zoologists especially have taken renewed interest in the Lamarckian view. And some of our plant ecologists hold to the idea that new species arise as the result of the gradual modification of existing species in

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response to environmental stimuli. However, this conception has been generally superseded by the mutation theory of the present day.

De Vries conceived the idea that new species spring full-fledged as it were from their parent species as a result of mutation, a sudden change in the germinal substance. He and other mutationists hold that natural selection plays an important rôle in evolution by eliminating at the very outset the great majority of mutations that are continually appearing in nature. It is now well known that germinal changes resulting in new mutant forms are of various orders of magnitude. It is also generally understood that most, if not all, mutations are caused by some sort of change in the chromosomes. For the sake of brevity I mention only the two general categories of mutations commonly referred to as *point mutations* and *chromosome aberrations*. A point mutation is an alteration of some sort, probably chemical, which changes only a single point or locus of a certain chromosome. A point mutation affects some specific character or characters of the organism, as for example, in the common vinegar fly, color of eyes or length of wings. Such are the mutations which made possible Morgan's brilliant analysis of the heredity of this fly and which in general cause those character differences which distinguish varieties within a given species. A chromosome aberration is an irregularity in the normally regular distribution of the chromosomes during cell division resulting in loss or duplication of whole chromosomes. If we assume that each chromosome contains several or many factors or hereditary units, we should expect the loss or duplication of chromosomes to exert a profound effect upon the soma of the mutant individual, and such indeed is the case. Classical examples are the three inconstant mutants derived by de Vries from Lamarck's evening primrose and now known to have 15 chromosomes instead of 14, which is the normal number for the parent species. Also there is a gigantic form derived from the same parent species, which has 28 instead of 14 chromosomes and

which is known as a tetraploid form, because its chromosome group contains four times the haploid or germ-cell number of the parent species.

In order to make clear the full significance of some of the results I am about to report, it may be well to explain how a tetraploid form might arise. A regular mechanism is present in all sexually reproduced organisms by which the functional germ cells or gametes receive just one half the number of chromosomes present in the somatic cells. The gametic number is, therefore, commonly referred to as the haploid or n -group and the somatic number as the diploid or $2n$ -group. Most important is the fact that the $2n$ -group, present in the somatic and primary germ cells, actually consists of n pairs of homologous chromosomes, and that in the reduction division preceding gamete formation the n pairs of chromosomes arrange themselves side by side on the equator of the division spindle and then the homologues separate and pass to opposite poles and so become segregated into different gametes. Incidentally, we may note that this separation of the homologues before gamete formation is just the mechanism required to explain Mendelian segregation, if we assume that Mendelian unit factors are carried in the chromosomes and this is now the generally accepted theory. With this mechanism in mind we can readily see how conspicuous mutants might arise through occasional irregularities in the normal mechanism of chromosome distribution. Thus, the failure of one pair of chromosomes to separate in the reduction division would result in one gamete with an extra chromosome which upon uniting with a normal gamete would produce an individual with an extra chromosome. If for some reason the reduction division were to be omitted entirely, gametes containing $2n$ chromosomes would be formed and reproduction by such gametes would result in a tetraploid or $4n$ individual. Again, in a tetraploid form, if the reduction division were to be suspended and the gametes containing $4n$ chromosomes were functional, reproduction would result in an $8n$ or octaploid individual. We now have good evi-

dence that one of the European species of *Crepis* with which we are working (*C. biennis* L.) is an octaploid species. It has 40 chromosomes and seems to have been derived from a species having 10 chromosomes (5 pairs) or 5 in the haploid group. Thus, we see that, while point mutations produce new varieties within a single species and while they might in time produce distinct new species having the *same* chromosome number as the parent species, the only mutations capable of producing new species whose chromosome numbers are different from those of the parent species are chromosome aberrations.

Another conception of the manner in which new species originate has recently been championed vigorously by Lotsy and others, who claim that all new species arise through natural hybridization between old species. Here again natural selection accompanied by mere chance must be assumed to preserve the occasional new forms which become established as species. Without going into detailed discussion of the deVriesian and Lotsyan theories of evolution, I will only point out that each has its limitations and that both together comprise only a portion of the whole truth about evolution. Point mutations alone can not explain the origin of species having different chromosome numbers, and chromosome aberrations seem to be so very rare that they could hardly suffice for an explanation of the origin of the thousands of species, genera and families of the sexually reproduced organisms. On the other hand, natural hybridization between species seems to furnish just the necessary means for the origin of new species with different chromosome numbers. But the impossibility of interspecific hybridization having been the sole method of evolution is obvious when we contemplate the simplest forms of life on the earth. The most primitive forms were doubtless incapable of sexual reproduction, so that new forms must have arisen by some sort of mutation. Thus, it appears that the origin of species, either by mutation or by the natural crossing of species, is only a part of the whole evolutionary process or, in other words, that the grand process of evolution depends

on the origin of new species both by mutation and by hybridization, accompanied by natural selection. This hypothesis seems likely to be verified by the results of our *Crepis* investigations. In fact, the experiments about to be described furnish strong evidence that some new species of flowering plants originate by mutation and others by hybridization.

HISTORY OF THE CREPIS INVESTIGATION

The researches of Morgan and others on the genetics of *Drosophila* furnished the inspiration for the investigations on *Crepis* which have been under way at the University of California since 1915. By that time something like 100 mutant characters had been discovered in *Drosophila melanogaster* and these had been shown to exist in four, and only four, linked groups corresponding to the four pairs of chromosomes present in this species. There was widespread interest in the *Drosophila* work at that time, but there was also much doubt and skepticism among biologists as to the generality of the chromosome theory of heredity as developed by the Morgan school. The *Drosophila* investigations are conducted in laboratories and the flies are reared in milk bottles. It was freely questioned whether the behavior of a species confined in such an artificial environment could be considered typical of other animal species, while the application of the theory to the plant kingdom was still further doubted. In order to make the necessary test with plant material, we were seeking a variable species with very few chromosomes when we found that two European cytologists had discovered that *Crepis virens*, or *Crepis capillaris* (L.) Wallr., as it is now known, has only six chromosomes! Application to various botanical gardens brought seeds of this plant as well as other species of *Crepis*. We found that *Crepis capillaris*, besides having so few chromosomes, is an annual reaching maturity within three or four months after sowing the seed, that the seeds require only a short rest period, and that the species is so highly variable as to be described in the

floras as polymorphous. We, therefore, set about the genetic analysis of this species and our first comprehensive report will be published in the near future. Meanwhile we found that certain other species of *Crepis* possess low chromosome numbers and that the genus contains about 170 species which are widely distributed throughout the world. We now have about 50 of these species under cultivation.

Meanwhile Morgan had called attention to the desirability of a crucial test of the chromosome theory of heredity and pointed out that such a test can best be made by crossing species that have been analyzed genetically as we are now analyzing *Crepis capillaris*. Investigators of *Drosophila* have striven for years to secure interspecific hybrids, but only one such hybrid has been obtained (*D. melanogaster* \times *D. simulans*) and it was completely sterile. Therefore, we soon began experiments on crossing different species of *Crepis* with the results I am about to describe.

As our familiarity with this group of plants increased our plan of investigation was expanded until now it is organized along three main lines: (1) Genetic analysis of *Crepis capillaris*, *C. setosa* and other species having but few chromosomes; (2) cultivation of as many as possible of the species in this genus accompanied by taxonomic study and cytologic investigation of chromosome number and individuality, for the purpose of evaluating the ordinarily accepted taxonomic characters in the light of cytologic and genetic data; (3) experiments with species hybrids with three objectives in view—first, to determine genetic relationships; second, to test the chromosome theory of heredity as suggested by Morgan; third, to throw light on the origin of species. I wish to take this opportunity to express my indebtedness to the loyal cooperation of my two colleagues, Dr. J. L. Collins and Dr. Margaret C. Mann, whose keen interest and originality of effort have made possible much of our progress to date, Dr. Collins conducting most of the breeding experiments and Dr. Mann doing all the cytological work,

while the writer is giving special attention to the taxonomic phase of the project.

TAXONOMY OF CREPIS

The plants now referred to this genus present one of the most difficult assemblages from a taxonomic viewpoint. *Crepis* belongs in the Chicory Tribe of the Sunflower Family and is closely related to *Hieracium*, so closely, indeed, that there are transitional species which can hardly be separated into the one or the other genus. In Engler and Prantl's *Pflanzenfamilien* *Crepis* is comprised of 11 sections or subgenera which differ more or less in characters considered important from a taxonomic viewpoint. For example, in the section *Eucrepis*, the species all have fruits without beaks, while in the section *Barkhausia*, all the species have beaked achenes. We are now cultivating species representing all but two of the 11 sections, and shall soon publish a paper on chromosome number and individuality as indicative of taxonomic relationship in these species.

CHROMOSOME NUMBER AND INDIVIDUALITY IN CREPIS

The chromosome numbers of 20 species have now been determined, and we find the following series of diploid numbers: 6, 8, 10, 12, 16, 18, 40. It is very unusual to find such a series within a single genus, the general rule being that a single genus contains only a certain number or multiples of that same number. This at once raises a question regarding the naturalness of the present grouping of species under *Crepis*. Some of the species studied are very striking in the individuality of their chromosomes. Thus, in *C. capillaris* there are three pairs, one long, one short, and one intermediate in length as was first shown by Miss Digby and Dr. Rosenberg. In *C. setosa* there are four pairs, of which two can easily be distinguished from *capillaris* chromosomes, because the shortest *setosa* chromosome is shorter than the shortest *capillaris* chromosome, while the longest *setosa* chromosome has a peculiar semi-detached portion; but the two

intermediate *setosa* chromosomes can not be distinguished from the intermediate *capillaris* pair. In *C. biennis*, which has forty chromosomes, they are all about the same length, so that they can not be definitely distinguished, but the longer chromosome of *setosa* can be distinguished from the *biennis* chromosomes.

SPECIES HYBRIDS IN CREPIS

Thus far we have succeeded in producing six interspecific hybrids as follows (numbers in parenthesis indicate the haploid numbers of chromosomes):

<i>C. capillaris</i> (L.) Wallr. (3)	× <i>C. tectorum</i> L. (4)
<i>C. setosa</i> L. (4)	× <i>C. capillaris</i> (L.) Wallr. (3)
<i>C. setosa</i> L. (4)	× <i>C. biennis</i> L. (20)
<i>C. setosa</i> L. (4)	× <i>C. aspera</i> L. (4)
<i>C. setosa</i> L. (4)	× <i>C. dioscoridis</i> L. (4)
<i>C. setosa</i> L. (4)	× <i>C. tectorum</i> L. (4)

The present paper deals with results from only the first three crosses. In each case the female parent is mentioned first.

CREPIS CAPILLARIS × CREPIS TECTORUM

There is but little to report on this experiment, inasmuch as thus far we have been unable to induce any first generation plants to grow beyond the cotyledon stage. This is contrary to what was expected because of the close taxonomic relationship between the two species. They are both found in the *Eucrepis* section and in fact they are quite similar, although sufficiently distinct to be universally considered as good species.

Cytological examination of cells in the root tips of these hybrid seedlings proved that they contained seven chromosomes as would be expected. However, the tissues of the growing point from which the stem should arise were badly disorganized. Apparently, there is something in the germ cells of these two species which makes them so incompatible that normal development of the growing stem is inhibited. As fertilization in the flowering plants is generally accomplished by the union

of one pollen nucleus *without cytoplasm* and the nucleus of an egg cell, it is fair to assume that the germinal incompatibility between *C. capillaris* and *C. tectorum* is due to some relation between the nuclear elements of which the chromosomes appear to be the most important. In short, the abortion of these hybrid seedlings is most reasonably explained as due to inability of the *capillaris* and *tectorum* n-groups of chromosomes to establish a properly organized cell nucleus. This is in harmony with the conception that the chromosome group functions as a single reaction system.

CREPIS SETOSA \times CREPIS CAPILLARIS

As these species have been placed in different and very distinct sections of the genus, it might be expected that hybridization would be impossible. Yet it was not only possible to obtain fertile seeds from this cross, but the first generation plants grew vigorously, set seed fairly well when crossed back to either parent, and may even be capable of producing viable seeds when self-fertilized, in spite of the high proportion of sterile pollen grains present in the anthers. The somatic cells contain seven chromosomes, two of which are easily identified as *setosa* chromosomes. The small proportion of viable pollen grains and low degree of self-fertility in the first generation hybrid are readily explained by cytological study of the behavior of the chromosomes during the two cell divisions just preceding formation of the pollen grains. The first of these divisions, in both of the pure species, is the reduction division at which the pairs of homologous chromosomes are separated into different cells and the second is an ordinary mitotic division. In these hybrids, as in species hybrids that have been studied previously by Federley and others, the reduction division is characterized by the fact that none of the chromosomes form pairs (see summary by Täckholm). This would be expected, of course, as none of the chromosomes are homologues, three of them being *capillaris* and the other four *setosa* in origin. The seven chromosomes merely arrange them-

selves in the equatorial region of the division spindle, and then proceed in a hit-or-miss fashion toward one pole or the other. The result must be the formation of gametes containing from 0 to 7 chromosomes in the following proportions, assuming that random assortment of the chromosomes takes place and that all potential gametes mature.

No. of chromosomes in F_1 gametes	Relative frequency of F_1 gametes
0	1
1	7
2	21
3	35
4	35
5	21
6	7
7	1

Now, if we assume that no gametes containing 0, 1 or 2 chromosomes are functional and that of the remainder only those will be functional that contain 3 *capillaris* or 4 *setosa* chromosomes, it is clear that only a small proportion of the gametes would be viable. Furthermore, by backcrossing an F_1 plant to *setosa* and counting the chromosomes of each plant thus obtained, the chromosome numbers in the F_1 gametes will be revealed. Thus far only five plants have been obtained by backcrossing the F_1 to *setosa* and these have chromosome numbers as shown below.

Relative frequency of F_1 gametes	Number of chromosomes in			Zygotes thus far obtained
	F_1 gametes	<i>setosa</i> gametes	resulting zygotes	
1	0	4	4	
7	1	4	5	
21	2	4	6	
35	3	4	7	1
35	4	4	8	2
21	5	4	9	
7	6	4	10	2
1	7	4	11	

The two offspring with ten chromosomes in their root tip cells are of special interest. Dr. Mann states that she

can identify in their chromosome groups two pairs of *setosa* chromosomes and one long one from *capillaris*. The identity of the other five chromosomes is still doubtful. These two plants are approaching maturity and when they produce flowers the behavior of the chromosomes in the reduction division will be studied with care as their behavior will show whether they are paired or unpaired. If these 10-chromosome plants contain 8 *setosa* and two *capillaris* chromosomes and are self-fertile, it should be possible by self-fertilizing them to produce two new forms that would breed true, one with 10 and the other with 12 pairs of chromosomes. Except that they would not have been tested as to their ability to maintain themselves in nature, they would really be new species. Even if these two 10-chromosome plants should not give the desired results, in time we shall obtain some that will do so. Thus it is clear that when the chromosomes are irregularly distributed, as in the reduction division of this species hybrid, a means is afforded by which chromosome number may be increased by one or more pairs. And in this way species with chromosome numbers not falling in a series of multiples of a single basic number would be produced. The unusual series of chromosome numbers found on the species of *Crepis* thus far counted may be explained in this way. Furthermore, if the F_1 hybrids derived from this species cross can be inbred, as now seems likely, it should be possible to observe the effects of one, two or three pairs of *capillaris* chromosomes plus a full set of *setosa* chromosomes on the characters of the plant. This will meet the requirements for a crucial test of the chromosome theory of heredity.

CREPIS SETOSA \times CREPIS BIENNIS

This cross also involves the two sections, *Eucrepis* and *Barkhausia*, since *C. biennis* has long been considered as close to *C. capillaris* and *C. tectorum*, yet the *setosa* \times *biennis* F_1 hybrid is even more self-fertile than the *setosa* \times *capillaris* hybrids. This is very remarkable, be-

cause the parent species differ so widely in their chromosome numbers. On this basis it is probably the widest species cross that has ever been made. As I shall soon explain, however, there is a special reason for the higher degree of fertility in this hybrid.

The *setosa* \times *biennis* F_1 hybrid has 24 chromosomes in its root tip cells, as would be expected, and two of them can be identified as *setosa* chromosomes. If the behavior of the chromosomes in the reduction division in this hybrid were similar to that which I have described in the *setosa* \times *capillaris* hybrid, all the 20 *biennis* and the 4 *setosa* chromosomes would go at random to either pole of the spindle and gametes would be formed containing all the way from 0 to 24 chromosomes. But such is not the case. To our surprise we found that the 20 *biennis* chromosomes form 10 pairs just before the reduction division in this F_1 hybrid, that the 4 *setosa* chromosomes remain unpaired, that the members of the 10 *biennis* pairs separate normally and then after they have moved part way toward the opposite poles the 4 *setosa* chromosomes pass in laggardly fashion and apparently at random toward either pole. This remarkable performance indicates that the 20 *biennis* chromosomes, although brought in by a single gamete, consisted of 10 pairs of homologues and suggests that *C. biennis* is at least a tetraploid species. We now have additional evidence that it is an octaploid species.

The classes of gametes formed by the F_1 *setosa* \times *biennis* hybrid, if there is random assortment of the *setosa* chromosomes will conform to the following series:

1	(10	<i>biennis</i>	+	0	<i>setosa</i>)
4	(10	"	+	1	")
6	(10	"	+	2	")
4	(10	"	+	3	")
1	(10	"	+	4	")

If these gametes all survive and unite at random in fertilization, the classes of F_2 combinations produced by self-fertilizing the F_1 hybrid will be:

1	(20	<i>biennis</i>	+	0	<i>setosa</i>)	all would breed true
8	(20	"	+	1	")
28	(20	"	+	2	") those with eleven pairs should breed true
56	(20	"	+	3	")
70	(20	"	+	4	") those with twelve pairs should breed true
56	(20	"	+	5	")
28	(20	"	+	6	") those with thirteen pairs should breed true
8	(20	"	+	7	")
1	(20	"	+	8	") all would breed true

It will be seen that among the F_2 progeny from this species cross 5 types should be found with no unpaired chromosomes and that these are expected to continue as constant races having 20, 22, 24, 26 and 28 chromosomes, respectively. Thus we anticipate the origin of five potential new species from a single species cross by means of self-fertilization of the F_1 hybrid plants. If these new constant races could again be crossed with *C. setosa*, other new types should be produced containing 5, 6, 7, 8 and 9 pairs of chromosomes, provided that we are correct in assuming that *C. biennis* is an octaploid species.

Preliminary observation on about 300 F_2 plants in the rosette stage show that they can be divided into a number of groups according to leaf characters and size of plant, such as the following: *biennis*-like, *setosa*-like, curly-leaved, linear-leaved, pineapple-leaved, broad-leaved ruffled, dwarf (including some of the preceding). One of the most interesting things about this F_2 variability is the possibility that it is due to the difference in number and identity of the *setosa* chromosomes. But there is also the possibility that it is due in some degree at least to recombinations among the *biennis* chromosomes. Recessive factors, which are present but usually masked by dominant allelomorphs in *biennis*, may become manifest in these hybrids. At any rate it seems certain that the new species which we expect to derive in this way will differ sufficiently in morphological characters to be easily distinguished without resort to examination of their chromosomes. But care will be taken to check the chromosome groups of all the distinct F_2 types derived.

By backcrossing the *setosa* \times *biennis* F_1 hybrid to *C.*

biennis it is clear from the F_1 gametic series given above that five types of progeny should be produced having 30, 31, 32, 33 and 34 chromosomes, respectively. Only two such plants have been examined cytologically, but they contain 32 chromosomes in their root tip cells and, what is more important, at the initial stage of the reduction division preceding formation of the pollen grains 15 pairs of chromosomes are present and the homologues separate from each other in regular fashion! This can only be explained by assuming that the wild *C. biennis* is actually an octaploid species, which means that its 40 chromosomes consist of 8 n-groups of 5 each. Whether we shall ever discover in nature the original species with 10 chromosomes (5 pairs), from which *C. biennis* seems to have been derived by repeated duplication, we do not know, but we hope to reproduce it by crossing some of our F_2 plants to *C. setosa*.

Additional reason for assuming octaploidy in the case of *C. biennis* is found in its comparatively large size and vigorous growth. It is much larger than any 5-paired species that we know. If *biennis* is really an octaploid species, the loss of the *gigas* characteristics would not be expected until the $2n$ condition had been reached. The F_1 hybrid of *C. setosa* \times *C. biennis*, having only 20 *biennis* chromosomes, is as large as *C. biennis* and resembles it closely. We await with anticipation the appearance of the plants that are to have only 10 *biennis* chromosomes.

RESUME

Our genetic analysis of *Crepis capillaris* has gone far enough to show that gene or point mutations cause polymorphism within a plant species. But such a process can never bring about the origin of species having new chromosome numbers. Our hybridization experiments with *C. biennis* have produced evidence that species with an increased number of chromosomes may originate through irregularities in the mechanism of chromosome distribution. Our results from crossing *setosa* and *capil-*

laris and *setosa* and *biennis* indicate that new species with different chromosome numbers may originate through hybridization of old species.

Thus we find evidence to support both the mutationist's and the hybridist's conception of the method of evolution. Natural selection among the individuals of new species that have recently arisen through mutation or hybridization must be hypothecated in a state of nature. The inheritance of acquired characters, especially the effects of the environment on plants, *may* act, separately or together with mutation and hybridization, as a slow but definite factor in evolution.

Finally, the results of these experiments indicate the possibility of still greater achievements by the breeders of economic plants. The improvement of crop plants through hybridizing them with related wild species has hardly been begun. Yet in combatting some of the worst diseases and pests of crop plants this is one of the most hopeful lines of attack. It is hoped that plant breeders throughout the world will be encouraged to concentrate both cytological and genetical study along this line, as it gives promise of results of far-reaching importance.

CROSSINGOVER IN THE SECOND CHROMOSOME OF *DROSOPHILA MELANOGASTER* IN THE F₁ GENERATION OF X-RAYED FEMALES

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IN two previous papers the writers have recorded "An effect of X-rays on the linkage of Mendelian characters in the second chromosome of *Drosophila melanogaster*" (Mavor and Svenson, 1923, 1924). It is of interest to determine whether this effect on crossingover, which consisted in an increase of the crossover value, is inherited. The experiment reported here was designed with this end in view. As in the case of the experiments reported in the papers referred to above the X-rayed and control females had the constitution (b pr c/+ + +); that is, they had the genes for black, purple and curved in one chromosome and the genes for the normal allelomorphs of these in the other chromosome. These heterozygous females were mated to males of the constitution (b pr c/b pr c); that is, the males were homozygous for black, purple and curved.

(a) CROSSINGOVER IN THE X-RAYED FEMALES

The X-rayed and control females were the F₁ of two pairs. In the case of those from the first pair "A" six were kept as controls and ten were X-rayed. In the case of the second pair "B" again six were kept as controls and ten were X-rayed. The technique used in these experiments has been described in one of the papers to which reference has been made (Mavor and Svenson, 1924). The X-ray dose was given at a distance of 12.6 cm from the tungsten target of the Coolidge tube with the current at 50,000 volts and 2.5 milliamperes. The time was 20 minutes. On our system of recording dosage this is rep-

resented by 32D. The X-raying was done on November 20 at 7 P. M. We are particularly interested here in the amount of crossingover in the fifth bottles, in which the X-rayed and control females remained from 7:00 P. M., November 26, to 7:00 P. M., November 27, that is, from the sixth to the seventh day after X-raying. It was from among the flies coming out in these bottles that the flies used in the next experiment were taken. The counts of the F_1 in the fifth bottles are given in Table I. In this

TABLE I

COUNTS OF F_1 IN THE FIFTH BOTTLES OF CONTROL AND X-RAYED FLIES

The X-rayed females were treated on November 20 at 7:00 P. M. and immediately mated. The Control females were mated at the same time. The X-rayed and Control females were in the fifth bottles from November 26, 7:00 P. M., until November 27, 7:00 P. M., that is, from the end of the sixth to the end of the seventh day after X-raying.

	No. of parents	Total F_1	Non- cross	Crossover			Percentage Crossingover	
				1	2	1-2	I	II
Control	6	823	654	26	139	4	3.77	17.38
X-ray	10	180	103	22	47	8	16.67	30.53

table it is seen that for the first region investigated, that between black and purple, the crossover value of the control females was 3.77 and of the X-rayed females 16.67, and that for the second region investigated, that between purple and curved, the crossover value of the control females was 17.38 and of the X-rayed females 30.53. These bottles, therefore, showed an increase in crossing-over in the X-rayed females similar to that already recorded in our previous papers. Since females were not bred from all the fifth bottles, Table II is given which shows the crossover values determined by taking only those bottles from which females were bred. This table shows that the bottles from which the flies were chosen gave almost exactly the same crossover values as those in Table I, obtained for all the X-rayed and control flies in the fifth bottles.

TABLE II

COUNTS OF THE F_1 IN THE ACTUAL BOTTLES FROM WHICH THE FEMALES WHOSE F_1 ARE RECORDED IN TABLE III WERE TAKEN

Four out of the 6 controls and 6 out of the 10 X-rayed females produced the F_1 recorded in this table.

	No. of parents	Total F_1	Non- cross	Crossover			Percentage Crossingover	
				1	2	1-2	I	II
Control	4	417	332	11	71	3	3.36	17.7
X-ray	6	157	90	20	41	6	16.6	29.9

(b) CROSSINGOVER IN THE F_1 OF THE X-RAYED FEMALES

In order to test whether this increased crossover value was transmitted to the next generation, females from among the F_1 of the X-rayed and control females recorded in Table II were bred. Females of the same con-

TABLE III

COUNTS OF THE F_2 OF X-RAYED FEMALES

These flies are the F_1 of virgin females of the constitution, $b\ pr\ c/+ + +$ from among the F_1 produced in the fifth bottles of the experiment and recorded in Table II. The parents, the crossingover in which is recorded in this table, remained in the first bottles from December 6 to December 9, and in the second bottles from December 9 to December 18.

Bottle	No. of Parent	Total	Non- cross	Crossover			Percentage Crossingover	
				1	2	1-2	I	II
Control								
1st	5	325	277	10	37	1	3.38	11.64
2nd	5	456	378	14	60	4	3.94	14.01
Total	5	781	655	24	97	5	3.72	13.07
X-rayed								
1st	14	654	521	18	105	10	4.28	17.58
2nd	14	821	719	21	73	8	3.53	9.88
Total	14	1475	1240	39	178	18	3.86	13.29

stitution as their mothers, that is, of the formula ($b\ pr\ c/+ + +$), were chosen and mated to males of the formula ($b\ pr\ c/b\ pr\ c$), that is, of the same constitution as their fathers. The same cross was, therefore, made in the case of the F_1 of the X-rayed and control females as was made when the X-rayed and control females were mated.

The results of breeding the F_1 of the X-rayed and control flies are shown in Table III. The flies were passed through two series of bottles and the crossover values are given separately for the first and second bottles. The total crossover values obtained by adding the counts of the first and second bottles show no perceptible difference between the crossover values for the F_1 of the X-rayed females and the F_1 of the control females. In the case of the F_1 of the control females the crossover value for the black to purple region was 3.72 and for the purple to curved region 13.07. The corresponding values for the F_1 of the X-rayed females were 3.86 and 13.29. In the first region, black to purple, no significant difference is seen in the crossover values obtained for the first and second bottles. In the case of the second region, purple to curved, the results are not so uniform, a noticeable difference being seen between the first and second bottles of the F_1 of the X-rayed and the F_1 of the controls. In this case the crossover value for the F_1 of the X-rayed in the first bottles, 17.58, is greater than that of the F_1 of the controls, 11.64, and the crossover value of the F_1 of the X-rayed in the second bottles, 9.88, is less than that of the controls, 14.01. It is doubtful, however, whether these differences when compared with the probable errors of the differences are significant. The difference divided by the probable error for the first bottles (F_1 of X-rayed compared with F_1 of control) is 3.99, and the same quantity for the second bottles is 3.05.

The conclusion to which this experiment leads is that the effect of X-rays on crossingover in the second chromosome is probably not transmitted, at least in anything like its original intensity, to the F_1 of the X-rayed females. This is in agreement with the results of Plough (1917) on the effect of temperature on crossingover in the second chromosome. He found (p. 166) that the effect of temperature, consisting in an increased crossover value, was not transmitted to the F_1 of the heat-treated females. The objection may, however, be raised that the F_1 females which were bred did not arise by crossingover

in the X-rayed females, having the same constitution as their mothers so far as concerned the characters used. Since crossingover did not occur in the eggs which gave rise to the F_1 tested, such eggs may have been unaffected by the X-rays and therefore the individuals arising from them would not in any case be expected to transmit an X-ray effect. The experiment ought, therefore, to be repeated, using F_1 of the X-rayed and control females which were crossovers, although of course here again the results would not be entirely free from the same objection, since even in these only a proportion of the crossovers owe their origin to the effect of X-rays.

The pressure of other work makes it unlikely that the writers will be able to repeat this experiment in the near future in a form which would make it conclusive so far as the transmission of the effect of the X-rays is concerned. The results are therefore submitted as they stand and the conclusion is drawn with the reservation considered.

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COUNTED GRAIN POLLINATIONS IN MATTHIOLA

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THE remarkable phenomena of inheritance of factors for flower form and plastid color in the Stock, *Matthiola*, as investigated by Miss Saunders (1911) are generally well known, and have already led to the suggestion that half of the pollen grains may fail to function. (*Cf.* Frost, 1915.) But it may be as well to recall such of the facts as here concern us.

Single stocks may be genetically of two kinds, pure singles or ever-sporting singles. The former breed true to singleness; the latter, when selfed, give a progeny of singles and doubles in the proportion of about five singles to six doubles. Of these, the doubles are sterile, and the singles are ever-sporting like their parent. This behavior is further explained by the results of crosses. The ever-sporting type is heterozygous for singleness (a dominant over doubleness), but whereas it forms ovules carrying either factor, its pollen all carries doubleness. If, then, the ovules are formed in the proportion of five single to six double-carrying, the observed results will follow. A further complication is that the factor or factor-complex for singleness does not occur in the same condition in the ever-sporting type as in the pure single. For in the cross, pure-single by ever-sporting, the heterozygote thus formed is not ever-sporting, but gives an F_2 with nearly a normal ratio of three singles: one double, thus showing that its single-carrying pollen is formed normally. Thus, the peculiar behavior of the ever-sporting type must be due to its singleness-complex, and for present purposes we may most simply make use of the notation used by Frost,

Pure single = SS
Ever-sporting = S's
Double = ss

The interest of the situation then lies in the fact that the distribution of factors amongst the ovules appears to be different from that in the pollen of the same plant. Such cases have been considered as due to "somatic segregation," but it is not clear how this conception would be applicable to the present case, even if such a process were possible. For we have here not the separation of two allelomorphs that thenceforward persist independently, as commonly understood by segregation, but rather the apparent loss of one allelomorph, the factor for singleness, on the pollen side of the flower.

Miss Saunders speaks indeed of an "elimination" of the singleness-complex before meiosis (1920, p. 184), and this could be reconciled with current views of chromosome mechanism and segregation at meiosis, if interpreted in the sense of a regularly recurring loss mutation somewhere in the cell-divisions leading to pollen formation. But even this means introducing a new hypothesis, which could be avoided if it could be supposed that both sorts of pollen are formed, but that at some stage the *S'* pollen fails to function, through the action of a gametic lethal. Accordingly, it was suggested to the writer by Mr. J. B. S. Haldane, who had devised a factorial scheme explaining remarkably the numerical results in inheritance of flower form and plastid color, but involving gametic lethals, that he should attempt to find out whether such were present in the pollen. The results so far obtained reveal a peculiar situation of some kind in pollen formation, and may be of interest as indicating a method of genetic research of which surprisingly little use has been made.

Under the microscope the pollen of both types of plant appears nearly all sound and uniform. It may conveniently be examined by mounting in potash and pressing on the cover-slip, when the contents will slip intact out of the spore-coats. There are a very few small badly formed grains. In tap water many of the grains germinate, but the tubes mostly burst soon afterwards. In 20

per cent. or 30 per cent. sucrose, almost all the grains germinate, and the tubes begin to grow. But since failure of certain pollen grains might occur at any stage of the processes between pollination and fertilization, it was decided to compare the pollen of pure singles and ever-sporters by counting out definite numbers of grains, placing them on the stigmas and determining the ratios of seeds obtained to grains applied, in the two cases.

The method was as follows: Glass needles were made, long and straight, by twice pulling out a solid glass rod in the blow-pipe flame—the second time with the flame very small. The pollen, of which each grain measures 30μ by 17μ , was spread thinly on a dry glass slide. Needles and a microscope were taken out to the flower bed. A needle was swept through the pollen on the slide, and then examined under the low power of the microscope. The grains taken up by the needle were thus easily counted, and if not too many were applied to the two lateral tufts of stigmatic hairs. The needle was then reexamined to make sure that it had given up all its grains.

The pollen was taken from anthers that had just opened in the bud. The buds of the ovule-parent were destaminated on the last day before the anthers would have opened, and pollinated when the stigmatic hairs looked most receptive—about 48 hours later.

The plants employed were grown from seeds of several races kindly sent by Miss Saunders to the Oxford botanical department. The number of grains placed on the stigmas of each flower was between 20 and 30; since the ovaries contain from 35 to 60 ovules, these would always be in excess. Had such low ratios of seeds to grains been anticipated, larger numbers of grains would naturally have been used. For ovule parents, ever-sporting plants were used throughout, as likely to give more critical results in the next generation. Care was taken that neither kind of pollen should be in any way favored as against the other. Pollen was taken from races of

equal vegetative vigor. Different flowers on the same raceme of the ovule-parent were usually pollinated alter-

TABLE I.

Pollen of pure singles		Pollen of ever-sporters	
d-cream by no-d-cream		d-cream by d-sulphur-white "R.H.K."	
Grains	Seeds	Grains	Seeds
22	9	24	0
22	0 (died)	24	0 (died)
22	1	26	0
23	4	24	0
25	2	20	0
20	0	20	1
23	0 (died)	23	0
25	9	20	0 (died)
20	2	22	0
26	2	22	0 (died)
24	0	28	0
24	0 (died)	28	0
27	1	20	0
22	1	23	0
Total	325	324	1

TABLE II.

d-sulphur-white CK by no-d-white		d-sulphur-white CK by d-sulphur-white CK	
Grains	Seeds	Grains	Seeds
28	8	24	1
25	0	25	0
23	1	24	0 (died)
		22	0 (died)
		25	0
d-sulphur-white CK by no-d-cream		d-sulphur-white CK by d-sulphur-white R.H.K.	
26	0 (died)	22	0
22	0 (died)	22	0
25	3	24	1
23	0		
23	2		
d-sulphur-white R.H.K. by no-d-cream		d-sulphur-white R.H.K. by d-sulphur-white CK	
25	3	23	0 (died)
d-red (family "19") by no-d-white		d-red (family "19") by d-sulphur-white CK	
26	0	24	0 (died)
24	1	25	0
26	0 (died)	23	0
		21	0
Total	296	304	2

nately with pollen of each kind, and all stigmas were, so far as possible, pollinated under the same conditions. The pollinations of Table 2 were made earlier in the year, when the routine had been less standardized. The work was much hampered by the cold and rainy summer of 1922.

The results are given in the following two tables. After pollination the ovaries in a few cases died, but usually persisted and grew to 20 mm or more even if they had set no seed. Ever-sporters are described as "d" and pure single as "no-d" plants.

Thus, in all, 621 grains of pure single pollen, distributed amongst 26 ovaries, gave 49 seeds, whereas 628 grains of pollen of ever-sporters, on 27 ovaries of the same ovule-parents, gave three seeds.

The ratios of seeds to grains were thus extremely low (1 to 12.7 for the pure singles), and possibly do not represent the chances of success of a pollen grain in normal mass pollination. For there may have been something unfavorable to seed-setting in the pollinations with small numbers of grains, either through ignorance of some essential factor, or possibly because the style and stigma may need to be brought into some favorable physiological condition by the stimulus effect of pollination with larger numbers. If the ratios obtained above held good, then in the case of "d" pollen, it would need about 8,000 grains to enable one flower to set a fruit nearly full of seed (as the sulphur-whites were found to do when left to self-pollinate naturally). This seems a lot, but calculations based on measurements of grains and of pollen sacs showed that each stamen contains about 124,000 grains, and each flower therefore 744,000. But the limiting factor will be the seating room on the stigma. However this may be, there still remains the question, why so great a difference between pollen of the two types. This might be due merely to a peculiarity of the two sulphur-white races used as pollen parents. But as against this, when selfed they set fruits nearly full of seed, while one of

them (RHK) was a strong-grower and a good match for the no-d-cream race. It is hoped to make further experiments, if necessary facilities can be obtained, with pollen from other "d" races, and also with "no-d" races as ovule parents.

But if the difference were really due to the different constitutions of "d" and "no-d" plants, then certain conclusions would follow. Since the difference is too great (being much more than 50 per cent.) to be due merely to elimination of S' pollen in the S's plant, the s pollen must also be weakened. But since, as mentioned above, in the Ss hybrid from the cross $SS \times Ss$, s pollen is known to be formed in nearly the normal proportion, it follows that its partial deficiency in the S's plant must be due to the constitution of the parent plant (unless to a direct lethal effect of the S' pollen upon it).

Whether the total elimination of S' pollen from the S's plant is partly dependent on the parental constitution, or due entirely to a gametic lethal, could similarly be determined if it were known whether or not s' pollen is formed in the s'S hybrid, from the cross $S's \times SS$. Experiments on this point are in progress.

For another method of counted grain pollination reference may be made to the work of Goodspeed (1918), on *Nicotiana*.

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THE PROBLEM OF PATTERN IN ORGANISMS¹

II. THE PHYSIOLOGICAL GRADIENTS

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THE PHYSIOLOGICAL GRADIENTS AS FACTORS OF PATTERN

Most organisms show some indications of a physiological axis or polarity. This means that not only the spatial, morphological order of parts and organs, but also the sequence of events in development and the physiological relations between parts are referable to certain directions in the protoplasm or cells composing the organism, or, geometrically speaking, to a line, the axis. With this axiate order is associated some sort of symmetry, either radial or bilateral, or some combination or modification of these. This means that in addition to the major or polar axis, we can distinguish other minor directions or lines, to which certain features of the order are referable. In fact, so far as its spatial characteristics are concerned, axiate pattern is referable to a tri-dimensional system of coordinates, in which the major or polar axis always differs in significance from the other two, while these may be alike and either indefinite or definite in direction, as in different sorts of radiate pattern, or they may differ from each other, as in bilateral pattern. In some organisms a simple axiate pattern persists throughout life; in others it is at first simple and becomes multiple, or minor axiate patterns of parts or organs arise in it in the course

¹ This paper, essentially in its present form, was written and accepted as a contribution to the proposed Williston Memorial Volume. Publication of that volume having proved impossible, the paper is dedicated to the memory of Professor Samuel Wendell Williston, both as an expression of personal regard and as a record of the purpose for which it was originally intended. The paper represents in considerable part the combined subject-matter of several addresses given at the University of Washington, the University of Wisconsin, the University of Cincinnati and elsewhere.

of development; again, the original pattern may be variously altered or modified during development, or it may even be replaced by another of different sort. In the higher animals the axiate pattern becomes exceedingly complex, the axes of parts, organs and cells showing all possible directions with respect to the primary axes.

Various lines of investigation show that the physiological axis in its simplest, most primitive condition is a gradient in physiological state, primarily quantitative in character, and involving a gradation in rate of the fundamental metabolic reactions, as well as in protoplasmic conditions. These gradients have been called metabolic, axial or physiological gradients. So far as investigation has gone, they have been found to be characteristics, not only of the major or polar axes of organisms, but of the symmetry axes as well, and also of the axiate patterns of organs and parts. Localization and differentiation in development occur in a definite relation to these gradients, the high end of the polar gradient, for example, becoming the apical end or head and the other organs along the polar axis arising at different levels of the gradient. In the bilateral invertebrates, so far as examined, the high region of the symmetry gradients becomes the median ventral, in the vertebrates the median dorsal region. In short, the system of axes to which axiate pattern is referable appears to be primarily a system of physiological gradients in the protoplasm. We may say that each point in the organism is determined and characterized by its position in the gradient system.

These gradients, however, are not necessarily present throughout the protoplasm of the body. The evidence indicates that they appear primarily in the superficial regions of the cell or multicellular body. In at least many protozoa and plant cells the gradients exist only in the superficial regions, and it is only these regions which show definite axiate pattern. But where definite internal organs with an axiate pattern exist, these also show physiological gradients, though both the original and the final

relations of these to the primary gradients differ in different organisms and according to the course of development. In many of the simpler organisms the primary gradients persist throughout life, but even in such forms they may undergo modification and complication in various ways. The primarily quantitative gradation usually gives rise in the course of development to a series of qualitative differences at different levels and the original gradient may disappear, leaving the effects and relations determined by it.

The existence of the physiological gradients is indicated or demonstrated in many different ways. A method of considerable value, particularly for the simpler organisms and earlier stages of developmental stages, is the susceptibility method. Extensive experimental investigation has shown beyond question that a general relation exists between physiological or metabolic condition in protoplasm and susceptibility to the toxic action of certain ranges of concentration or intensity of at least many if not all external agents. To certain ranges of concentration or intensity above the limit of tolerance or acclimation, susceptibility varies directly with rate of fundamental metabolic reactions. In such concentrations or intensities the higher levels of a physiological gradient are earlier, or more completely inhibited in growth and development, or die earlier than less active levels. In certain lower ranges of concentration or intensity which permit acclimation or recovery after temporary exposure, the higher levels of a gradient undergo the processes of acclimation or recovery more rapidly or more completely than lower levels. In other words, the more active protoplasm is more susceptible to extreme conditions and more capable of adjusting itself to less extreme conditions than the less active.

The susceptibility method may be used in various ways. The differential susceptibility along a physiological gradient may be indicated by differences in survival time, or, under somewhat less extreme conditions, by dif-

ferences in degree of inhibition of growth or development or motor activity, and under still less extreme conditions, or after temporary exposure, by rate or degree of acclimation or recovery. Through the differential susceptibility of different levels of a physiological gradient it is possible to alter the course of development in certain definite, predictable ways, determining great differences in size and proportions of parts, or even their presence or absence. Unquestionably a large proportion of teratological forms, appearing in nature as "accidents," result from differential susceptibility at different levels of the physiological gradients of developmental stages.

The existence of the gradients is also indicated by differences in rate of penetration of various substances, *e.g.*, vital dyes, by differences in enzyme activity, in rate and amount of reduction of KMnO_4 , and in electrical potential. In certain forms it has also been possible to demonstrate directly that the gradients are gradients in rate of oxygen consumption and CO_2 production. All these physiological differences at different levels of the gradients disappear when the organism is killed or soon afterward.

In many forms the gradients are indicated by structural gradations along the axis, such as a gradation in cell size, as in many animals and plants the protoplasm-yolk gradation in many animal eggs, the degree of vacuolation in many plant embryos, etc. The gradients also appear in the rate and sequence of development along the axes. In axiate animals development at first proceeds most rapidly at the high end of the polar gradient, which becomes the apical end or head, and in the high region of the symmetry gradients, the median ventral region in the bilateral invertebrates, the median dorsal in vertebrates.

The primarily quantitative differences at the different levels of a physiological gradient serve as the basis for determining the qualitative differences which arise in differentiation. This may occur in various ways. For example, a difference in the relation between intake of nu-

trition and the rate of oxidation at different levels may determine the accumulation of certain substances in the protoplasm at one level and their absence at another. In many yolk-bearing animal eggs the yolk appears chiefly or only at the lower levels of the polar gradient where oxidation is least rapid. Again, differences in concentration of certain substances at different levels may determine different reactions and different products and so initiate differentiation. Moreover, in so complex a system as protoplasm differences in state of colloids, enzyme activity, electrolytic dissociation, water content and various other factors at different levels of a gradient may also be factors in the initiation of differentiation. As soon as differentiation begins, definite and orderly chemical correlation becomes possible and plays a part in further differentiation.

DOMINANCE AND SUBORDINATION IN RELATION TO THE GRADIENTS

Experimental investigation has shown that a relation of dominance and subordination exists between different levels of a physiological gradient. In general, any level is to some extent dominant over lower levels and is dominated by higher levels and the high end of the gradient dominates all lower levels within a certain distance. This means that a given level of the gradient affects physiologically lower levels of the gradient to a greater degree than they affect it. This is merely a special case of the general rule that a more active region of protoplasm affects less active regions more than they affect it. All the evidence at hand indicates that this relation of dominance and subordination depends primarily, not upon the mass transportation of substances from one level to another, but upon the transmission of the energy changes which constitute physiological excitation. It depends essentially upon the fact that a higher level of the gradient behaves with respect to a lower level like a region of excitation with respect to an unexcited or less excited

region. Dominance appears, not only in the functional relations along an axis, but in the developmental relations as well. It has been demonstrated experimentally for *Planaria* and some other forms that localization and differentiation of organs along the polar axis are physiologically determined primarily in the direction from anterior to posterior and not in the opposite direction.

Physiological dominance is limited in range and its range varies with the activity of the dominant region and the condition of the protoplasm through which the transmitted change passes. When we decrease experimentally the metabolic activity of a dominant region, *e.g.*, the growing tip of a plant, the apical end or head region of an animal, its dominance decreases in range, or may disappear, if the decrease in activity is sufficient. Under these conditions changes may occur at lower levels which did not take place as long as the dominance persisted. In plants, for example, new buds may appear, or buds previously inhibited may grow, and in the simpler animals, where differentiation has not progressed too far, similar reproductive processes may take place. These are cases of physiological isolation. In general, physiological isolation has been shown to occur in four ways: By increase in size of the body beyond the limit of dominance and consequent isolation of parts most distant from the dominant region; by decrease in activity of the dominant region; by blocking the correlative factor in its passage through the protoplasm; and finally by increasing the activity of a subordinate part to such an extent that it is no longer subordinate. It has also been shown that physiological isolation is the primary factor in at least many processes of agamic reproduction and reduplication of parts, and it is probably concerned in all such processes.

Physiological dominance is, in fact, one expression of the physiological gradient. The attempt has been made recently to show that a very direct physiological continuity exists between the physiological gradients and the structural and functional relations of the nervous system

in animals. To sum up, the physiological gradient in its simple form, both as regards the differences in physiological condition at different levels and the relation of dominance and subordination, shows all the characteristics of an excitation-transmission gradient. In fact, the only marked difference is that the physiological gradient is relatively permanent as compared with the ordinary excitation-transmission gradient. But whether this similarity has any real significance can be determined only by investigation of the origin of the physiological gradients.

THE ORIGIN OF THE PHYSIOLOGICAL GRADIENTS

Our knowledge along this line is still very incomplete, but various suggestive and some very conclusive facts are at hand. In the first place, physiological gradients are determined in various organisms by a quantitative differential in the action of an external factor. In such cases the external factor either localizes a region of high activity, essentially a region of excitation, and the gradient arises from this, or the differential action on different regions of the protoplasm may directly determine the gradient. In the eggs of certain species of the alga *Fucus* the physiological axis, which has been found to be a gradient, is determined by the differential action of light, the most strongly illumined region becoming the apical pole of the plant. In the *Equisetum* spore the physiological axis is similarly determined, and the determination of dorsi-ventrality in plants by the differential action of light is a familiar fact.

In pieces or cell-aggregations of the simple animals, *e.g.*, sponges and hydroids, new polarities may be determined by differences between a free and an attached surface, the free surface becoming the apical and the attached, the basal pole. In other cases an apical pole and so a new axial gradient may be determined directly by localized injury which gives rise locally to high metabolism and rapid growth and so determines the localization

of a dominant region. According to Lund a new physiological polarity may be determined in hydroids by means of the electric current and the facts show that in such cases a new physiological gradient is determined. In still other cases, such as the localization of adventitious buds in certain plants and the development of new axial gradients in hydrozoan embryos and pieces after experimental obliteration of the preexisting axes slight chance differences in activity between different cells or cell groups are sufficient to determine the new dominant regions and so the new gradients and the whole course of development of new individuals.

In various forms among the lower invertebrates the free pole of the growing egg becomes the high end of the polar gradient and the apical or anterior end of the embryo. The actual determining factor in these cases is unknown, but it may be a differential in oxygen supply or in CO_2 removal or both. In the higher animals, where the respiratory exchange is effected chiefly through the blood, the circulatory relations of the growing egg appear, at least in some cases, to be a factor in determining its gradient. In most plant eggs also the polarity is apparently determined by a differential relation to the parent body. Further experimental work is necessary for the determination of the particular factors of the environmental relation which are concerned in the origin of egg-polarity.

As regards the origin of symmetry in development, particularly bilaterality, our knowledge is still less complete, except in the matter of its relation to light or other external factors in plants. It is possible to obliterate symmetry experimentally and to determine different sorts of symmetry in the same protoplasm and a symmetry gradient may be substituted experimentally for a polar gradient, but as regards the origin of symmetry in embryonic development in animals we know but little. In certain cases it is perhaps determined by the spermatozoon, in others perhaps by the position of the matura-

tion spindle before it moves to the apical pole or of the first cleavage spindle or some other division.

In some cases of agamic reproduction the physiological gradients of the new individual are determined anew in response to local conditions, but in others the old gradients persist as such through the reproductive process and the axes of the new individual are determined by these gradients. In such reproductive processes the de-differentiation and acceleration of metabolism involved in the reorganization of the part into a new individual bring the gradients up to the physiological levels characteristic of other individuals. In these cases the gradients, and therefore the axial relations, are directly inherited as gradients from the parent individual. To what extent such inheritance of the axial gradients from earlier cell generations occurs in eggs is uncertain, but that it may occur is of course possible. It should be pointed out, however, that such persistence or inheritance does not involve Lamarckian assumptions. There is in such cases no transmission or transfer of something from the body to the germ, but merely a persistence of certain physiological conditions through several or many cell generations. When the planarian body undergoes fission or is cut into pieces, the axial gradients usually persist and the axes of the new individual are the same as those of the individual from which it came. If inheritance of axial gradients in eggs occurs, it is likewise simply a persistence of physiological conditions previously determined and in no sense a Lamarckian inheritance. On the other hand, the gradient conception may provide a physiological basis for certain phenomena which are often interpreted in Lamarckian terms.

Whether or not the axial gradients persist through reproduction, the evidence indicates that they arise in the first instance as a response to a differential action of a factor external to the protoplasm concerned. The external factor determines directly or indirectly a high rate of activity in some region or a differential in rate in

different regions and the excitatory changes, transmitted with a decrement from the region of highest activity thus determined, give rise to an excitation-transmission gradient. If the action of the external factor continues for a sufficient length of time, more or less permanent changes in protoplasmic conditions are brought about, corresponding to the different degrees of excitation at different levels. These changes constitute the substratum for a more or less permanent gradient and undoubtedly determine its further development after the external factor has ceased to act. The physiological gradient then originates essentially as a differential excitation in response to action of an external factor, but when once established through the occurrence of protoplasmic changes which are not readily reversible, its further development is independent of the external factor, but is of course determined by the specific hereditary constitution of the protoplasm in which it exists.

According to this conception axiate pattern originates as an excitation-transmission pattern which determines more or less permanent protoplasmic changes and so becomes the basis of the developmental pattern. The relation of dominance and subordination is primarily the relation between higher and lower levels of the gradient and constitutes the basis of the functional relations which attain their highest development in the nervous system. As differentiation in relation to the gradient occurs, it affords a basis for orderly and definite chemical or transportative correlation, and this becomes increasingly complex and important as differentiation progresses and channels of transportative communication develop.

THE PROBLEM OF SURFACE-INTERIOR PATTERN

The cell is primarily an organism, though it may be integrated with other cells into an organismic pattern of larger scale. There are also certain organisms, *i.e.*, bacteria, which appear to be even simpler than cells of the

usual type. In most unicellular organisms and even in some of the still simpler organisms axiate pattern undoubtedly exists, but apparently the most general feature of pattern in these simple organisms is the completely radial or spherical symmetry with differences between surface and interior. This surface-interior pattern is apparently a more generalized and more primitive pattern than the axiate, in that the only differences are those between the surface as a whole and the interior. Any mass of protoplasm exposed to the action of environmental factors must acquire some sort of surface-interior pattern, since conditions at the surface are different from those in the interior. It seems probable that surface-interior pattern is not fundamentally different from axiate pattern, except that in the former the differential is between the surface as a whole and the interior, while in the latter a differential between different parts of the surface exists. But since we can reach the interior only through the surface, it is difficult to determine whether a physiological gradient, originating in excitation-transmission relations from surface to interior, is present in surface-interior pattern, or to what extent the material exchange between the protoplasm and the external world is concerned in determining this sort of pattern.

The cell and at least many organisms simpler than cells apparently represent primarily surface-interior pattern. The localization and differentiation of the nucleus was probably originally determined by conditions in the interior of the mass of protoplasm. It is, in fact, difficult to conceive how the nucleus as a definite organ could have arisen in any other way. Moreover, there is no reason to believe that the persistence of the nuclear substance from one cell generation to another and through varying conditions is entirely independent of the conditions in the interior, as distinguished from the surface of the cell.

The presence of axiate pattern does not mean the disappearance of surface-interior pattern. In fact, surface-

interior pattern exists in all organisms, whether unicellular or multicellular. Axiation and symmetry represent merely new differentials superimposed on the more general surface-interior differential. The surface-interior pattern is of course complicated and modified by the presence of an axiate pattern, but still exists and must exist in some form in any mass of protoplasm exposed to an environment, and all living things are so exposed. It is the most general and most primitive organismic pattern, and at least certain of its features are obviously not inherent in the protoplasm, but are directly dependent on exposure to the action of external factors. For example, the appearance of a plasma membrane or an ectoplasmic layer is a direct result of exposure of protoplasm to environment. This is perhaps the most general structural characteristic of organismic pattern and the first step in organization, and if this is dependent upon external factors, the preformistic conception is discredited at the outset.

As in the case of axiate pattern, the particular kind of surface-interior pattern which arises in any given case depends primarily upon the specific hereditary constitution of the protoplasm concerned. The relation to external factors merely determines that surface-interior pattern of some sort shall be present. The character of surface-interior pattern may differ widely in different protoplasms, even in the same environment, but this means, not that the pattern is inherent, but rather that the potentialities of behavior in a given sort of organismic pattern are primarily dependent on the nature of the material on which the pattern is imposed.

ORGANISMIC PATTERN AND THE CHROMOSOMES

Whether or not we regard cell pattern as primarily of external origin, recent investigations and current theories make it necessary at least to inquire concerning the significance of the chromosomes for organismic pattern and particularly for the pattern of multicellular organ-

isms. Certain lines of recent work in cytology and genetics have led to the postulation of definite, more or less stable chromosome patterns, differing for each chromosome, and some biologists believe that the totality of chromosome patterns in the nucleus of the germ cell represents in some way, or constitutes the basis of the organismic pattern which appears in development of the individual.

It is, however, a well-established fact that in general every cell of the multicellular organism possesses the full complement of chromosomes and therefore supposedly the complete nuclear pattern of the fertilized egg. Nevertheless, different cells or cell groups become different and different kinds of organismic correlation arise between them. Recognition of this fact led Boveri years ago to admit that local conditions, rather than the nuclear pattern, must be responsible as activating or determining factors for the origin of these differences. In other words, Boveri held that organismic pattern must be of external origin. More recently, however, this question has either not been considered, or the fact has not been recognized that cells which are originally alike can not of themselves become different. Loeb, in "The Organism as a Whole," merely states the problem when he says that "the egg is the embryo in the rough" and does not attempt to show how the egg has attained this condition. Similarly, Morgan, in a recent discussion of the organism as a whole, does not account for the fact that cells, which are originally alike as regards nuclear pattern, become different in orderly and definite ways.

As Conklin, F. R. Lillie and others have shown, various eggs possess more or less definite cytoplasmic patterns, but if we maintain that these patterns are determined by the nuclear or chromosomal pattern and that the pattern of the multicellular organism results from the distribution of the components of this pattern among different cells in development, we have merely formulated the problem of organismic pattern in the preformis-

tic terms of nuclear pattern, and the problem of nuclear pattern remains. Moreover, this conception involves still another difficulty. If nuclear pattern is the fundamental factor in determining the cytoplasmic pattern and if the cytoplasmic pattern of the egg represents the primary pattern thus determined, as this conception apparently assumes, we should expect each cell which arises from the egg with a full complement of chromosomes gradually to approach the cytoplasmic pattern of the egg, even though different cells originally received different components of the egg cytoplasm. In short, since they all possess the same nuclear pattern, the different cells should become more and more alike, instead of more and more different. But, since they do become more and more different, it must be assumed, either that cytoplasmic pattern, once established, may continue to exist independently, or in spite of nuclear pattern, or else that such cytoplasmic pattern may influence and alter nuclear pattern. Either of these assumptions amounts to abandonment of the original hypothesis of nuclear or chromosomal pattern as the determining factor. It is evident that, even when we start with the cell conceived in terms of the theories of nuclear pattern, something more is necessary, *viz.*, the action of an external factor, to account for the pattern of the multicellular organism.

Beyond question the nuclear substance is of fundamental importance in relation to the specific hereditary constitution of the protoplasm of a particular species. I have tried to show, however, that this specific constitution represents, not organismic pattern, but the material or substratum on which this pattern is superimposed as a physiological order which merely determines what potentialities shall be realized and where each realization shall occur. There is no necessary conflict between this conception of organismic pattern and the theories of chromosomal pattern. Whether the chromosomes shall finally prove to be relatively simple fluid crystals, or linear series of definitely localized "factors," or something dif-

ferent from either of these, it remains true that, so far as organismic pattern is concerned, they represent merely potentialities. For the realization of different potentialities in different cells or cell groups, all of which originally possess all the potentialities, a pattern on a larger scale is necessary, and this pattern must be of external origin.

THE NATURE OF GROWTH¹

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I

ONE of the most conspicuous characters of organic beings is their power to grow—to become what, at a previous stage of their existence, they were not. The acorn becomes an oak tree, the helpless babe becomes an Aristotle. What is the cause of the transformation? What forces are involved in these non-reversible processes which we term development, or growth? The oak tree can not be put back into the acorn, neither can the man be put back into the baby. It is needless to say that much of our philosophy of the universe has arisen from the attempt to answer questions which man has asked concerning these phenomena. In biology there have been two distinct schools—the vitalists, who held that the processes in organic beings are qualitatively different from those occurring in other forms of matter, and the mechanists, who hold that the differences, if any, are merely quantitative. The vitalistic naturalists have attempted to explain the organism in terms of a soul either connected with or separate from some superior soul. The mechanistic naturalists have attempted to explain the organism in terms of mass, time and space. It is not necessary to judge the successes or failures of the two schools in the present discussion. I do feel constrained, however, to remark that the students of the growth process (especially the growth of plants) have busied themselves with the irrelevant and unimportant aspects of the subject and have neglected most of the problems which might explain

¹ Read at a symposium on "Growth and permeability," held by the Pacific Division of the Plant Physiological Section of the Botanical Society of America, Los Angeles, September 19, 1923.

growth. In the words of a recent article by Kidd and West:²

A few fundamental principles are necessary for the study of growth and development. These are conspicuous by their absence in existing text-books of plant physiology, which excel in the assemblage of interesting curiosities and of uncorrelated details. The phenomena of *normal growth* seem to call for further study and analysis and for the application of mathematical treatment.

In view of the great diversity of organic life and of its complex manifestations, it is not surprising that biology should be one of the last of the sciences to abandon the grab-sample methods of study with which man originally tackled the problems of the universe.

It is my purpose to show how the problem of growth may be quantitatively studied by the application of methods already in use in other departments of science and how their use simplifies the problem. I think it fair to assume that most of us have come to the realization that it is necessary to extend the application of quantitative methods in the study of physiology. In addition to measuring the factors of the environment, we must measure the response of the organism. Of the many responses of organisms, the two of paramount biological importance are growth and reproduction, yet their study has been long neglected.

II

One of the first results of the application of quantitative methods to biological processes is the discovery of their continuity. The organism of to-day is the organism of yesterday plus or minus a certain number of molecules of water, carbohydrate, *et cetera*. The processes of hydration and dehydration which play such an important part in the changes of carbohydrates, proteins and fats are mainly brought about by oxidases, reductases and other enzymes. These processes go slowly in comparison with many chemical reactions and are often reversed, but they are the principal centers of metabolic activity in the organism.

² *Ann. Appl. Biol.*, 6: 2, 1919.

Studies of growth phenomena should eventually lead to a consideration of the energy relationships involved. We can not but believe, in the absence of contrary evidence, that the growth processes of living organisms are manifestations of the energy relationships prevailing in other parts of the universe. The study of these energy relationships ought not, therefore, to be disregarded. Our aspirations for such knowledge, which have received so much encouragement from the discovery of the principle of the conservation of energy, can never be entirely suppressed.

For purposes of this discussion the major phases of growth will be considered to be enlargement, differentiation and senescence. The organism which begins as a single cell becomes larger. As it becomes larger, its parts become differentiated. The little seedling is originally little more than a slender rod. But the rod grows and becomes differentiated into shoot and root, and the former undergoes further differentiation into vegetative and reproductive organs, each of which may become highly complex in structure and function. These processes constitute development or growth. Following them come changes which betoken the end of the growth process, leading to senility and eventually to death.

III

If we concur in the view that growth is an increase in size accompanied by a differentiation in members, we may inquire concerning the factors which are related thereto.

The organism acquires materials from its environment and by means of them becomes larger or different. Water is one of the substances which is acquired in large quantities and is of general importance for vital processes. A plant's ability to grow is, therefore, highly dependent on its ability to absorb water. The structure of most organisms is such that considerable water must pass through them every day. The lack of no other sub-

stance (oxygen excepted) is more quickly or keenly felt than the lack of water. These statements are so evident and so trite that you are perhaps impatient with me for taking the time to make them. My purpose in introducing them is to call attention to the dependence of growth upon imbibition. MacDougal³ has unquestionably shown that imbibition is dependent upon the colloidal state of the cell and that the amount of water imbibed depends as much upon colloidal state as on access to an adequate supply. From MacDougal's experiments it is evident that proteins have the greatest water capacity in acid solutions and that pentosans show the greatest degree of hydration in neutral or slightly alkaline solutions. Miss Carey⁴ has shown that gelatin absorbs more CO₂ after soaking in water and still more after soaking in HCl solution than the fresh gel absorbs. This may be due to the fact that gelatin absorbs more water from acid solutions than from pure water. Slight changes in the conditions cause conspicuous changes in the rate of imbibition. The ions of the common constituents of soil solutions have varying effects upon the imbibition and swelling of protoplasm and other colloidal substances. The studies on this question are legion, but they too often concentrate their attention on the water and ions which go through the cell colloids, whereas the importance belongs to what stays in them. In the case of root cells of a terrestrial plant the transmitted substances are obviously important because from them the rest of the plant acquires many of the necessary materials.

IV

The growth of an organism usually begins at a slow rate, gradually increases for a time, then becomes slower until it stops. The same is true of an autocatalytic reaction. It begins slowly, but as more of the catalyzing substance is produced, the reaction proceeds at an in-

³ MacDougal, D. T., Carnegie Inst. Publ., 297, 1920.

⁴ Carey, C. L., *Physiol. Res.*, 2: 407-432, 1923.

creasingly rapid rate. As the supply of reacting substances is used up, and as the products of the reaction increase in amount, the reaction slows down and comes eventually to a stop.

Robertson⁵ has shown that the rate of a reaction like this may be expressed by the differential equation

$$\frac{dx}{dt} = kx(A - x)$$

When integrated this becomes

$$\log \frac{x}{A - x} = K(t - t_1)$$

In these equations x = size of the plant at time t ; A = final size of the plant; t_1 = time at which half the final size is reached; k = a constant; and $K = Ak$.

This equation has been used to express the growth of sunflowers,⁶ of pear shoots,⁷ of cucumber leaves,⁸ of lemon shoots,⁹ and of white rats.¹⁰ The production of flowers on the cotton plant,¹¹ of ammonia by ammonifying bacteria,¹² and of carbon dioxide by yeast¹³ follow the same equation. The results of these and of other investigations have shown the general applicability of this equation to the study of the growth of organisms. Considering the nature of the materials studied, it must be said that the equations give results of satisfactory accuracy.

V

Our next question may well be: "What do these equations teach us concerning growth?" "Are we to regard

⁵ Robertson, T. B., *Arch. Entwicklungsmech.*, 25: 581-614, 1908.

⁶ Reed, H. S., and Holland, R. H., *Proc. Nat. Acad. Sci.*, 5: 135-144, 1919.

⁷ Reed, H. S., *Jour. Gen. Physiol.*, 2: 545-561, 1920.

⁸ Gregory, F. G., *Annals of Bot.*, 35: 93-123, 1921.

⁹ Reed, H. S., *Proc. Nat. Acad. Sci.*, 7: 311-316, 1921.

¹⁰ Reed, H. S., *AMER. NAT.*, 55: 539-555, 1921.

¹¹ Prescott, J. A., *Annals of Bot.*, 36: 121-130, 1922.

¹² Miyabe, K., *Soil Science*, 2: 481-497, 1916.

¹³ Rippel, A., *Ber. deut. bot. Ges.*, 37: 169-175, 1919.

them as evidence of physiological principles, or merely as evidence of successful jugglery?" Some physiologists maintain that the resemblance between observed and calculated values is entirely superficial and without significance.

This is an important question, and no effort should be spared in attempting to answer it. It seems to me that we are at the beginning of a new epoch in plant physiology and that we have a responsibility in orienting our ideas to the wider view we are gaining.

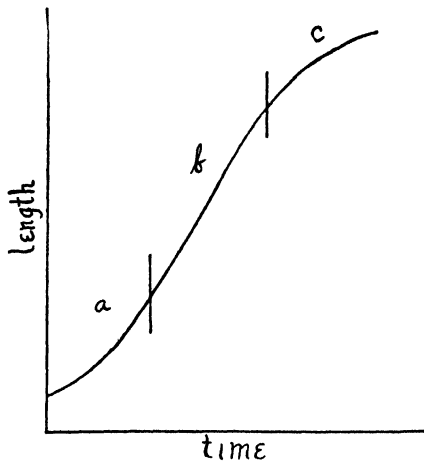


FIG. 1. The growth of pear shoots calculated from the equation

$$\log \frac{x}{114-x} = .0242 (t - 47.4)$$

The S-shaped curves (Fig. 1) representing the growth of an organism may be divided into three parts. The lower part (*a*) represents the initial period in which the size increases rather slowly; the middle portion (*b*) represents the period of most rapid growth; and the third portion (*c*) represents the period commonly known as the period of senescence.¹⁴

Since the curve plotted from the equation represents the growth of a wide range of organisms, we must guard against giving it too narrow an interpretation.

The growth represented by *a* undoubtedly is the result of an increase in the number of cells when they increase

¹⁴ Priestley, J. H., and Pearsall, W. H., *Annals of Bot.*, 37: 239-249, 1922.

at a geometrical rate (the growth is an exponential function of time), while the supply of raw material in the environment is greatly in excess of the absorbing capacity of the organism. In the case of yeast and bacteria the number of cells is small in proportion to the volume of the nutrient medium. In the case of the higher plants, the young seedling or young shoot first draws on accumulated products of metabolism of the parent plant, gradually supplementing them with products synthesized in its own tissues. In the case of mammals, the young are utilizing during this period the nutrient fluids of the mother, which are especially rich in growth-promoting materials, and are gradually supplementing them with food from other sources.

The portion of the curve represented by *b* covers the period of most rapid growth and is the most conspicuous to the observer. Many physiologists who have studied growth have confined themselves to this part of the growth process. During this time the increase in size is almost (but not exactly) proportional to the time, *i.e.*, the cells increase (in size or number) at an arithmetical rate. We may assume that during this period the catalyst of growth is abundant, but that the growth is conditioned upon the rate at which the organism can obtain from the environment the materials upon which the catalyst can work. During this period growth is more apt to be influenced by fluctuations in the external medium than in the other two periods. In many cases growth during this period must depend upon the rate of oxygen absorption, and in the case of green plants, upon the rate of carbon-dioxide absorption. In all cases it is closely conditioned upon the rate of water absorption, and may therefore depend upon the rate at which acids are produced.

The last portion of the curve covers the period of senescence. The growth during this period comes to a point where no further increase in size takes place; as a matter of fact, some organisms may shrink because of the loss of water. The portion of the curve represented

process. The amounts may be for some time so small that they have no material effect upon the catalyst, but, as time goes on, they accumulate and gradually inactivate the catalyst. Their retarding action may be increased or decreased by changes in the external medium whereby the absorption of oxygen or of other substances is altered, but the effect of external conditions on the form of the growth curve is not as great as generally assumed.

VI

The concept of growth-inhibiting substances throws much-needed light on the problem of cyclic or periodic growth. Many, if not most, organisms have more than one growth cycle. The intra-seasonal cycles in the growth of shoots of certain fruit trees are well marked.²² Studies on the fluctuating growth of apricot shoots²³ produced evidence that their growth rate was alternately increased and diminished by some factor which varied harmonically during the growing season. So far as the evidence now in hand permits, it seems logical to assume that cyclic growth is related to the accumulation of some growth-inhibitor of a colloidal nature which inactivates the catalyst. After a time the physical or chemical state of the growth-inhibitor is altered, or the amount of catalyst present is in excess of the inhibiting power of the materials, and a new cycle of growth is begun.

VII

This discussion has dealt primarily with the equation for autocatalysis

$$\log \frac{x}{A-x} = K(t - t_1)$$

without any intent to ignore the other equations which have been used to express the growth of organisms. For some purposes Wilhelmy's equation,

$$x = A(1 - e^{-kt})$$

²² Reed, H. S., *Jour. Gen. Physiol.*, 2: 545-561, 1920.

²³ Reed, H. S., *Natl. Acad. Sci. Proc.*, 6: 397-410, 1920.

is well suited to express the growth curve. Blackman²⁴ has shown that for short intervals the growth of a plant may be expressed by the compound interest formula

$$W = W_0 e^{rt}$$

where W = dry weight of the plant at time t , W_0 = initial dry weight of the plant, r = the rate of interest, or "efficiency index" of dry-weight production, and e = the base of the natural logarithms. It is evident that $r = \log_e W - \log_e W_0$.

Mitscherlich²⁵ proposed the formula

$$\log (\sqrt[n]{A} - \sqrt[n]{y}) = \log \sqrt[n]{A} - c \cdot x$$

where n = a variable indicating the probable number of environmental factors, A = maximum possible dry weight, y = dry weight at time x , the time x being expressed in vegetative periods of arbitrary length.

It is not my present intention to discuss the relative merits of one or another of these equations but to stress the importance of expressing growth as an orderly dynamic change in which increase in size is equal to an exponential function of time. He who wishes may discard any or all of the equations mentioned, but there is no escape from the conclusion that the growth of plants should be studied as a problem in chemical mechanics.²⁶

VIII

Without attempting to discuss additional data we may stop now to inquire what we may conclude from the facts already presented.

Primarily, it seems that growth is a dynamic process which causes enlargement and differentiation in organisms. It seems to me that these facts show a continuity in the growth process which has hitherto been unappreciated by physiologists. If we will replace our former ideas of the haphazard character of growth by the idea

²⁴ Blackman, V. H., *Annals of Bot.*, 33: 353-360, 1919.

²⁵ Mitscherlich, E. A., *Landw. Jahrb.*, 53: 167-182, 1919.

²⁶ The reader interested in a critique of various equations should consult Schüepf, *Ber. deuts. bot. Gesell.*, 38: 193-199, 1920, and Rippel, *Jour. f. Landw.*, 70: 9-44, 1922.

that it is a slow chemical reaction of the first order, our ideas will more nearly accord with the observed facts. This may be nothing more than exchanging the idea that organic beings are the sport of the gods, for the idea that they are the result of a divine harmony achieved through the operation of natural laws.

The question which is often raised by those who have been accustomed to think of the organism in terms of its environment is, "How does the organism manage to maintain such an even growth rate in spite of the myriad fluctuations in its surroundings?" This applies especially to plants. Animals having the power of movement can, to some extent, get out of unfavorable surroundings, but the higher plant is fixed, and must take the environment as it finds it. We must admit that raising or lowering some essential factor, such as heat, to the death point or near the death point, will so alter the growth rate as to invalidate the foregoing assumptions. But, unless the conditions become too nearly lethal, organisms show a constancy in their development which merits our attention and study.

Our understanding of the behavior of the organism will be clarified if we apply the theorem of Le Chatelier, a theorem which has been derived from physico-chemical conceptions. It may be stated as follows: If a system in equilibrium is subjected to a constraint by which the equilibrium is shifted, a reaction takes place which opposes the constraint, *i.e.*, one by which its effect is partially annulled. In all cases, whenever changes in the external condition of a system in equilibrium are produced, processes also occur within the system which tend to counteract the effect of the external changes.

Let us see if we can apply this theorem. A plant growing in the field is in dynamic equilibrium with its surroundings, otherwise it is dead. If cloudy days or drought or other changes ensue, the activities of the plant take such a course that the effect of the changed conditions is minimized, and the growth rate generally suffers

little change. This appears to be an integration of the activities of the plant which results in its specific response—a response which is far more dependent upon the inherent nature of the plant than upon the surroundings.

I am aware that many, if not all, of the views here expressed will be criticized, and often adversely, in the next few years. If there be anything of truth in them, it will be sifted out. Undoubtedly I have erred in the direction of over-simplification, but this is due to an attempt to lay aside, so far as possible, all irrelevant matters and to discuss growth simply as a problem involving slow transformations of material at a rate proportional to time.

CONCERNING THE HOLLOW CURVE OF DISTRIBUTION

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IN his recent book, "Age and Area," J. C. Willis advances a number of interesting hypotheses concerning the origin, growth and differentiation of species, genera and higher groups, and, particularly, the spread of species geographically. In the development of his theories he leans very heavily upon the deductions drawn from a statistical treatment of certain phases of systematic biology. The graphical plotting of nearly or quite all such data, so far as it involves frequency of occurrence as contrasted with size (in its most general sense), gives rise to the so-called hollow curve. It is this particular phase of his work to which I desire to call especial attention.

This paper is not presented as a criticism of any of Willis's theories but simply to call attention to some of the curious features presented upon a more or less complete analysis of this curve. The constant occurrence of this characteristic hollow curve primarily aroused my interest in this problem and my first motive was to try to determine to what extent, if any, it carried a meaning to the systematic biologist. If the curve does express some actual biological fact or facts in a useful form, it would be well worth while for it to be more generally understood and utilized. My own convictions as to its value are as yet somewhat unsettled. Certainly, it seems to bear considerable significance in certain cases, at least.

I am particularly indebted to Professor G. F. Ferris, of Stanford University, for valuable suggestions and criticisms as well as for aid in the gathering of most of the data presented herein. I am greatly indebted to Mr. Carl D. Duncan, of Stanford University, for reading the manuscript and for many helpful suggestions and criticisms.

As Bateson has already stated in his review of "Age and Area," there is some difficulty in following Willis in his arguments and conclusions, since nowhere does he give a clear unembellished statement of his hypotheses and the reasons for believing this particular curve to be of such profound importance as confirmatory evidence of them. This difficulty may be due more to the enveloping abundance of evidence than anything else. Consequently, as a preliminary to the results of my own investigations, I am stating his propositions and giving what I interpret to be the substance of his reasons for their support.

Briefly, the proposition in the first case is this. If we study the fauna or flora of any given geographical unit, we find in *all* cases that more genera are represented by but a single species than by two species; more with two than with three and so on. Then, if we plot the frequency with which the genera of various sizes appear against their absolute size (using, in this and other cases, the number of included species as a criterion), we obtain the hollow curve in its typical form (frequency to size).

Secondly, if we classify any group of plants or animals according to the geographical extent of their range (as determined by the most outlying stations), we similarly find more species occupying small or limited areas than medium or large areas. Plotting the data again gives us our hollow curve (frequency to area).

Thirdly, we find a more or less close correlation between the area over which a genus ranges and its size. Thus, it is found that genera of limited geographical distribution tend to be small, particularly monotypic to tritypic. Genera which are wide ranging, on the other hand, tend to be large, *i.e.*, to include many species. Taken on the average, the concordance is remarkably close. The curve obtained is very close to or identical with the usual hollow curve (area to size).

Fourthly and finally, if we take any given group of organisms without regard to faunal or floral restrictions, and plot the frequency with which genera of certain size

appear, we again obtain the hollow curve, monotypic genera being most numerous, ditypes next, then tritypes and finally a straggling group of larger genera (frequency to size). This proposition is essentially the same as number one.

As Willis emphasizes at every point, the mechanical regularity with which the hollow curve appears seems to call for some more or less mechanical explanation. With this in view he advances a number of hypotheses to explain certain phenomena here made apparent for the first time. He also and primarily utilizes it as support for his theory of age and area, which was first and principally based upon a study of the relationship and distribution of endemic floras, and a very good if not conclusive demonstration of the essential slowness of biotic geographical dispersal. In the following paragraphs, then, I shall give a bare statement of the most important of these hypotheses or explanations, but without any of the abundant supporting evidence given by Willis in his book.

The statement of his law of age and area will give us with essential completeness his ideas concerning the first two propositions outlined above. It is quoted verbatim from "Age and Area."

The area occupied (as determined by its most outlying stations), at any given time, in any given country, by any group of allied species at least ten in number, depends chiefly, so long as conditions remain reasonably constant, upon the ages of that group in that country, but may be enormously modified by the presence of barriers, such as seas, rivers, mountains, changes of climate from one region to the next, or other ecological boundaries and the like, also by the action of man and by other causes.

To him the approximate correlation between the size of the genera and the extent of territory over which they range means that on the average species and genera of small or limited distribution are, phyletically speaking, young, while wide-ranging species and genera are on the average much older. The fact that so many of these "young" forms occupy such relatively minute areas as is shown by a study of the endemics of Ceylon and New

Zealand is advanced as "proof" that species arise by mutation as contrasted to the theory of evolution by infinitesimal variation, which theoretically requires a large area for specific and generic birth. Relic genera and species are believed to comprise but a small minority of the total number of forms of limited geographical distribution.

Another conclusion, and to me, so far as this study is concerned, the most interesting one, is that species and genera must arise more or less mechanically and regularly to give rise to such a curve. Likewise, the spread of species must on the whole approximate, in *related* groups, a more or less constant rate of dispersal.

To begin with we must remember, however, that the mere conformance of a curve derived from actual data to the curve postulated by a theory does not positively prove the thesis. It is in the nature of negative evidence. In other words, as we already know from a study of other curves, there may be more than one hypothesis whose theoretical curve will coincide with that derived from actual data.

The essential postulate that a species will occupy less territory when young than when considerably older is one which I think few will deny. It is not this, then, which is open to question, but whether the correlation between age and area is maintained with sufficient accuracy to enable the use of one known factor to deduce the other is decidedly more so. In other words, while admitting the theory of age and area as fundamentally true, can we believe that in the face of the innumerable environmental factors of a modifying nature, particularly barriers, that the correlation will be maintained to any recognizable extent? Willis maintains and ably defends this thesis, and this is largely the essence of his theory. The strongest point in his argument so far as this problem is concerned, then, is the close correlation obtained (as a whole and on the *average*) between the absolute size of genera and the area over which they range.

In any case a second hypothesis, as ably and logically defended, has not, as yet, been forthcoming and hence in any work I have done toward amplifying or modifying his conclusions, I am assuming for this purpose its fundamental soundness. In this connection I wish definitely to state that I am not committing myself *positively* to any of the conclusions arrived at. They are merely presented as more or less interesting possibilities or in some cases probabilities, with the hope that they may stimulate others to pursue this line of investigation further and determine more or less accurately their true value.

Willis's work was based almost exclusively upon plant studies. Mine are almost exclusively upon animals, and it is possible (although I think scarcely probable) that Willis would object to authorizing such a wholesale transference of his theories and conclusions. There is, however, so far as I have been able to discover, no difference between the two. It is possible that the age or size to area correlation would not hold so closely with animals as with plants, but even this appears doubtful to me in the light of my investigations.

While admitting the sound points in his arguments on the one hand, we must not overlook a considerable number of difficulties and criticisms which the theory must surmount on the other. Of these the first in my opinion is the *quality* of our systematic work. Is the quality and completeness of our systematic work sufficient for the drawing of any very far-reaching conclusions on the basis of the data which it supplies? For example, one of the most puzzling features about the whole problem was the appearance of apparently identical results from all types of work, and consequently from all types of data, good, bad and indifferent. That this similarity is not quite as great as it superficially appears is one of the points I hope to establish further on.

In this connection let us consider the Coccidae or scale insects in relation to Willis's fourth proposition, *i.e.*, frequency to generic size. Probably almost every worker

in this group will agree that the existing classification is but a very poor expression of the actual biological conditions within the group. It is quite safe to say that very few of the genera within this group as it stands at present are actual expressions of biological fact. It is of course true that in part genera are subjective creations and have, to a certain extent, no "actual" existence in nature and consequently there is apt to be wide divergence of opinion as to their limits. Yet in spite of this, there is doubtless considerable basis in actual biological fact for genera. This is not a place to enter far into a discussion of the Coccidae, but some illustrative examples may be given.

In Figure 2-B is plotted the curve as based upon the Fernald Catalogue of the Coccidae for the world. This

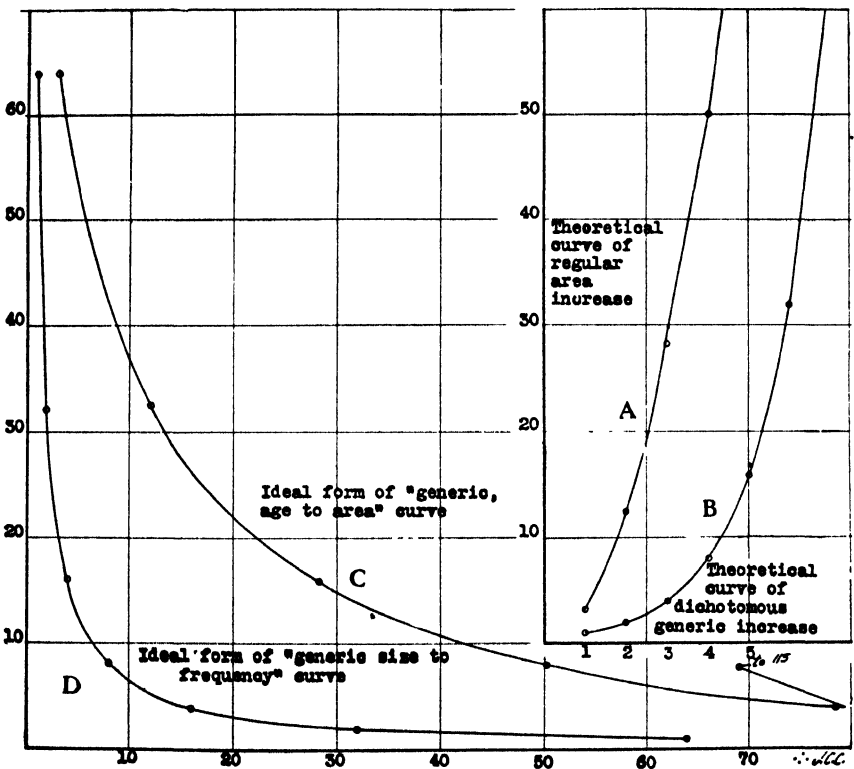


FIG. 1, A-D. "Ideal" curves of generic-specific and area increase. In A and B, the ordinate represents increase, the abscissa time. In C and D the abscissa represents size or area; the ordinate frequency.

catalogue is now over twenty years old and was conservative for its day. Consequently, the curve as obtained is an extremely conservative one and is based upon sets of genera, very few of which are now regarded in anywhere near the same light as they were at the time the catalogue was published. An analysis carried through the whole group will show actually a very profound modification of the genera, and yet the hollow curves from the data obtained from such an analysis would be, apparently at least, no different.

Such a rearrangement has actually been made in part, by MacGillivray. He goes far to the other extreme, and in his book, "The Coccidae," over 100 new genera are named. From this volume the curve shown in Fig. 2-H was constructed. As may be easily seen, the superficial appearance of these two curves is striking, although the MacGillivray curve is decidedly unbalanced, particularly toward the monotypic genera.

Hence we are moved to inquire whether data drawn from such sources are of any particular value. It seems odd that we should get even similar results from two such discordant sources, and it appears as though some factors other than those of a purely biological nature are at work.

From this it was seen that some standard basis must be devised for the comparison of such curves. For this purpose, what I am calling the ideal curve was obtained in two independent ways. In the first place, the hyperbolic appearance of the curve suggested that the simple formula of $XY = K$ would satisfy the conditions. As a corollary to this it is obvious that the number of *species* in any given generic class should theoretically be equal to the number of *species* in any other generic class. Thus, if we have a group with 100 monotypic genera we should theoretically have 50 ditypic genera, 33 tritypic genera and finally a single genus of 100 species. Consequently, the ideal curve for any group under consideration may be obtained by simply totalling the number of species in-

cluded and extracting the square root of the sum. (This is the method by which all the ideal curves in the accompanying figures were obtained.) This root, then, is equal to the K of our formula and determines the limits of our curve.

The second derivation of the ideal curve was deduced from purely theoretical conditions with the following ideas as guides:

(1) Other things being equal, phyletic differentiation and spacial distribution will vary directly with time.

(2) The ideal curve for any type of phenomena may be obtained by divesting it of all modifying factors and carrying the progression to its logical mathematical conclusion.

Thus, in order to obtain the simplest possible expression for the evolution of genera (or species), we may assume an original genus which gives rise by mutation to a new genus for each regular time unit or interval, which rate is effective, also, as applied to the mutant or daughter genera. This is a simple case of geometrical progression, mathematically expressible by the formula, $a + (a + 1)^1 + (a + 1)^2 + (a + 1)^3 + \dots$, in this case " a " being equal to one. It is the type of increase displayed by a regularly dichotomously branching diagram or by the reproductive activities of a single-celled organism increasing by simple binary fission. In other words, we have simply expanded this type of reproduction to account for the origin of genera and species. This parabolic curve is shown in Figure 1-B.

Then, by assuming that species arise at the same rate and by the same method outlined above for the genera and carrying our progression for convenience' sake to the number 64 and plotting the sizes of the genera obtaining at this particular point we find that the eight values we obtain coincide exactly with points on the ideal form of the curve, as determined by using 64 as our constant or curve limit in our first method. This curve, with the points obtained by this hypothesis indicated, is shown in

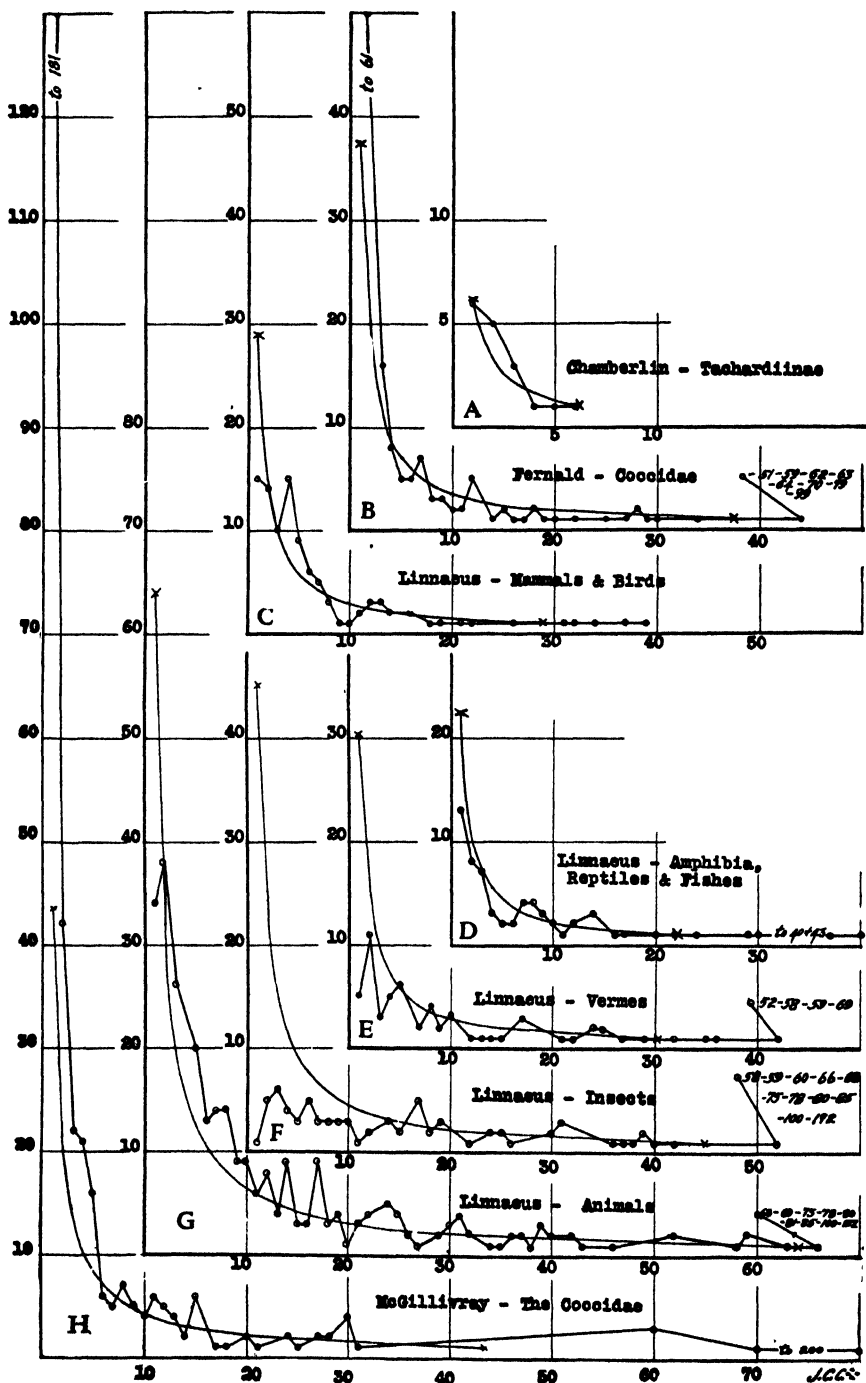


FIG. 2, A-H. Various hollow curves contrasted with their "ideals." In all cases the ordinate represents frequency; the abscissa, generic size.

Figure 1-D. This close correlation, then, seems to entirely justify the use of such a curve as a standard for our purpose.

To make clear this last method of obtaining the ideal curve we may carry out our progression as follows: 1—2—4—8—16—32—64. Then, assuming the same method and rate to hold for species, it is obvious that the original genus will now contain 64 species, there will be two genera with 32 each, 4 with 16, 8 with 8, 16 with 4, 32 with 2 and 64 with 1 species each. Thus it is clear that what we have here are simply the whole non-fractional frequency values on an $XY = K$ curve, with 64 as a curve limit.

In assuming our ideal curve it must be borne in mind that it is here advanced purely as a standard for rational comparison and *not* as a pattern.

To continue our comparison of the Fernald and MacGillivray curves, using our ideal curves as standards of comparison, we find that what seemed at first a strongly marked similarity is now no more than a more or less superficial resemblance, although even now sufficiently striking. The most obvious difference is in the enormous preponderance of monotypic genera in particular and small genera in general in the MacGillivray curve. It lies almost wholly above the ideal curve for such a number of species and there are nowhere near the "proper" proportion of larger genera. The Fernald curve very closely approximates its ideal, the only radical departure being in the slightly excessive number of monotypic and ditypic genera.

Now what seems to be the only reasonable conclusion we can draw from our study so far is this. While both these classifications are actually and seriously far from the truth, they are nevertheless of sufficient accuracy to show the underlying evolutionary tendencies of the group. On the other hand, they certainly can not be relied upon for detailed analyses.

For example, if we could assume that MacGillivray's data were 95 per cent. accurate, instead of (as a pure

guess) 75 per cent. or probably even less, it would be possible more or less safely to interpret the enormous preponderance of small genera, together with a paucity of large ones, as indicating that we had here a very rapidly evolving group and one which is comparatively young or else one that is probably senescent. On the same basis Fernald's curve would indicate a comparatively stable group—possibly near the maximum of the group's development.

Before going any further it might be well to note some other factors which also operate to give us the hollow curve. As we have already demonstrated, the theory of "mechanically regular" origin of species and genera will alone account for the curve. On the other hand, there is no reason for supposing that there are no other factors which could give the same result. In other words our curve may be, not the resultant of a single factor, but the combined effect of several. Willis discounts the possibility of relics or remnants of dying genera influencing his results to any extent. As before mentioned, he believes that, as compared to the enormous mass of newly evolving forms, relics will simply form a very small minority of the total. On the other hand, if we take a large group of organisms which has evolved to a racial maximum and final extinction, it is certainly beyond question that every species evolved in that group must have ultimately become extinct in one way or another, and consequently at some time or other must have posed as relics. In other words, the two are equalities. This objection to Willis's theory, however, is not as serious as it might at first sight seem. Thus, while there is no doubt that once a group has reached an evolutionary maximum and *racial* extinction has set in, we should expect (were all differential evolution brought to a standstill), that purely as a matter of chance a random killing out would maintain the curve unchanged to the final phase of extinction. On the other hand, such random killing would undoubtedly not occur and certain groups would become

extinct far earlier than other hardier ones. Nor can we assume a cessation of divergent evolution in a senescent group. We know as a matter of actual fact that right up to the final phase of their extinction, the ammonites and trilobites as well as other now extinct groups produced new genera, and as a matter of fact were probably evolving new generic types with as great if not greater rapidity than at any other time in their history. (Using, of course, as criteria, the same type of morphological characters for limiting genera arising in this final period before the extinction of the group that were applied to the genera existing before this "running wild" of generic and specific characters, as a final phase of their senescence had appeared. This proviso is necessary to obviate possible irregularities introduced by a sudden shifting of our generic concept.) Then the inevitable dying out of genera—either through actual extinction of included species or their transformation into new generic types (whether or no this latter occurs is questionable, the former not so) would tend inevitably to produce an increasingly greater and greater proportion of monotypes. Thus, large genera as a class would inevitably disappear first, *i.e.*, be reduced to smaller and smaller genera through the dying out of some of their members and metamorphosis of others, so that up to the final phase of their extinction we should have our curve not only maintained but accentuated. And as may be seen by a consideration of the above the preponderance of monotypes would or at least could be "new" beginners. From a study of our curve alone there would be some difficulty in determining whether or no we were here dealing with an adolescent or senescent group.

On the basis of our ideal curve, if we plot the *percentages* of monotypes we find them varying inversely with the size of the group under consideration. Thus the ideal curve for a group of one included species would show 100 per cent. monotypes; of four species, 50 per cent. monotypes; of nine species, $33\frac{1}{3}$ per cent. monotypes; of

16 species, 25 per cent. monotypes, and so on. In this connection the significant thing to observe is that these are all points on an ideal curve with 100 (per cent.) as a curve limit. Thus, it is obvious that the larger groups will have relatively the smallest proportion of their species included in monotypic genera.

It is interesting and significant in this connection to note that the monotypes plotted as percentages, against time, will, as evolution proceeds from birth to senescence and death, inevitably result in a double or descending and ascending hollow curve. Thus, if we assume a group arising monophyletically from a single genus and species, we have originally 100 per cent. of monotypes. This percentage will decrease in a descending geometrical series as differentiation and phyletic increase becomes effective, and the minimum percentage of monotypes will be attained at the maximum development of the group as a whole. Then, as the process of extinction begins, the percentage of monotypes will increase until, as the final phase of extinction is reached, we will have a single genus and species remaining or 100 per cent. of monotypes.

In brief, the minimum *percentage* of monotypes in any given group of organisms will be attained at the point of its evolutionary maximum of abundance.

Thus, if our period of observation of any given group could be extended over a sufficient lapse of time to determine in which way the general trend of monotypic percentage varied, we could say for certain whether or no we were here dealing with a declining or ascending group.

Then, with some other considerations in mind, such as ecological adaptability and geographic range, we could from a knowledge of the percentage of monotypes make some logical estimate of how far, in a relative time sense, our group is from either a maximum of phyletic development or from death.

If these deductions are true, they will enable us to understand why groups appear to arise with such sudden-

ness as is witnessed by the paleontological record and why they as quickly disappear, though they may have in between these two phases an enormously long period of comparative group stability.

From the above considerations it is seen that the relative percentage of monotypes possessed by any given group is in effect an index to the phylogenetic development of that group.

In consequence of the above considerations, it may be easily seen that our data must be of a considerably more accurate type than is the case with those given by either MacGillivray or Fernald. The peculiarities of these particular curves may be purely fortuitous.

As a third factor, tending, in practice, to produce a preponderance of monotypes, may be cited the methods of our systematic work. Comparatively few genera are given birth to, systematically speaking, with their full complement of species. In other words, our systematic knowledge is in large part very incomplete, so that many genera, as we now know them, will in the future undergo profound modification through the addition of newly discovered species and redefinition of generic limits. Large genera, as soon as they become unwieldy, are subdivided or split, a tendency certainly present and one which has been much deplored by some.

Is not this, however, exactly what actually occurs in nature? For example, we may be sure that certain characters, all of potential variability, possessed in common by an original group of species, will tend less and less to parallel each other's development as the group grows. This is, of course, a direct consequence of the specialization of these original characters plus their individual and independent "acquisition" of new features. In other words, we have our old group breaking down into new ones, or more accurately attaining a super-group rank. For example, two species may well possess 50 significant characters in common, but the chance of such a group now grown to include 50 species, possessing this same

series of parallelling characters, is very small. This "breaking down of genera" probably occurs in an inverse geometrical ratio to the size of the genera, being greatest in the larger ones. This is, of course, merely another expression of our previously stated theories of generic growth and differentiation.

To recur to our discussion. While the method of our systematic work will no doubt tend to produce a preponderance of monotypic genera to begin with, we may assume on the other hand that after a certain preliminary stage has been reached our data will assume the nature of a random sample and hence this factor will become negligible or non-existent. In this connection it is probable that comparatively few groups, among the animals particularly, have reached this stage. This is especially true among the insects, where we find that to date the majority of our species are known from very few records.

As a matter of fact there is no doubt that more species in our entomological literature are known from one record than from two, more from two than from three, and so on. In other words, we obtain our typical hollow curve, which in this case undoubtedly is a graphical representation of our systematic knowledge of the group and not of its phylogeny at all.

To get back to the main thread of our study. In order to determine just where we begin to get significant indications of our hollow curve, an analysis of the *Systema Naturae* of Linnaeus for the animals was undertaken.

The curve for the work as a whole was first plotted (Fig. 2-G). Then, to complete the study, the book was divided into four sections, each of which was considered separately. In the first of these curves, that for the birds and mammals (Fig. 2-C) (which group we may safely assume Linnaeus understood better than any other), we get a very good approximation to the ideal curve for the group, except for the fact that monotypes to quatrotypes are present in almost equal numbers. In the second curve, that for the reptiles, fish and amphibia (Fig. 2-D)

we have an even better correlation, since here we actually obtain a preponderance of monotypes, although the curve as a whole does lie below its ideal. The curve for the vermes (Fig. 2-E) and for the insects (Fig. 2-F), the other two classes considered, show scarcely a trace of the underlying "hollow" nature of the curve. We know of course that Linnaeus's actual classification of the vermes and the insects was undoubtedly more artificial than for the mammals and birds. The most surprising thing is the comparative "excellence" of the fish-reptile curve. Linnaeus surely had no more real comprehension of them than he had of the mammals and birds, and it seems probable that the approximation here obtained is due largely to chance, and possibly to the relatively greater incompleteness of his knowledge. The almost total obliteration of the curve for the vermes and insects is almost surely entirely due to his *real* lack of appreciation of their actual relationships.

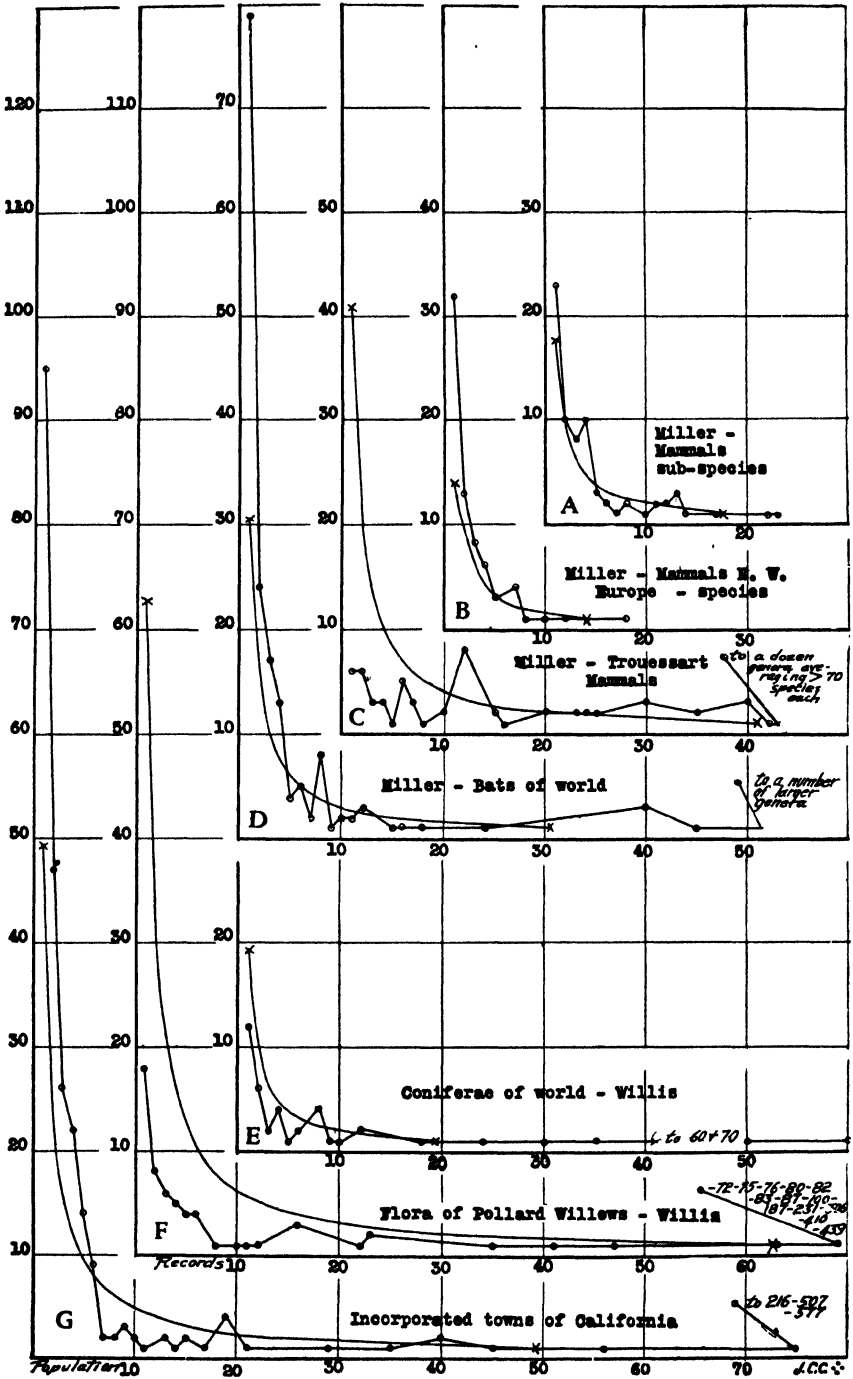
Knowing the actually crude nature of his work (whatever its relative quality or value) it is surprising to note the fairly close resemblance of the Linnæan curves as a whole to the typical hollow curve form. However, as we may easily see, when compared to their ideal forms, the resemblances are largely superficial. Thus, all these curves (excepting the anomalous fish-reptile curve) are very irregular, they lie almost wholly below their ideals and the number of ditypic genera is greater than the monotypic ones. An obvious interpretation is that here we are dealing with incomplete and highly inaccurate data.

Then to determine the type of curve given by systematic work of the very highest quality, an analysis of Miller's Catalogue of the Mammals of Western Europe was made. Three curves were derived from the data given in this work. One of these recognizes subspecies (Fig. 3-A), one species only (Fig. 3-B), while the third and last is a curve in which the genera represented in Europe were plotted against their total (instead of just

the European) number of species contained in them. This latter curve is based upon Miller's own estimates as given in his catalogue, supplemented in small part by Trouessart's catalogue of the Mammalia. This latter curve is shown in Figure 3-C. A very interesting result is manifested. The curves based upon the species and subspecies, relatively, are essentially the same, the only noticeable difference being a small reduction in the number of small genera in the subspecific curve. The Miller and Trouessart curve, however, exhibits a very striking modification in that there is an almost total obliteration of the "hollow" in the curve. The monotypes (and some of the other smaller genera) obviously represent, in this case, the truly endemic or new genera of Europe, and as we see they are actually very few. The "humps" at six and twelve are misleading. They are primarily due to Miller's method of estimating the number of included species as "a half dozen" or "about a dozen."

This flattening of the curve is most striking and might indicate several things. The most obvious meaning would appear to be that we have here a territory which is comparatively new to invasion, or else one that is having its fauna rapidly exterminated, probably the former. The "newness" is demonstrated by the actually small number of precinctive genera and by the comparatively large numbers of genera here represented by one or two species, which in adjacent regions reach an extraordinary degree of abundance. Thus the region would appear to have something in the nature of a recent "overflow" fauna, obviously resulting in a preponderance of apparently small genera when the European species alone are considered. Then in addition to the above effect we have our curve somewhat accentuated by a number of undoubtedly dying genera.

It appears that this Miller-Trouessart effect should be of considerable practical value in the balancing and limiting of faunal and floral areas and life zones, inasmuch as



the curve gives us a simple graphical method of weighing our data.

As may be seen at a glance the superficial resemblance of the Miller curves to the Fernald curve, for example, or (neglecting the ideal comparison curve) to the Linnaean fish-reptile curve is sufficiently close to enable us to state positively that only in the most general sense will the quality of the data affect the form of the curve, whether given alone, or contrasted with its ideal form.

In further substantiation of the above statement we may note the form given by plotting the genera of the bats of the world as given by Miller in his treatise on the "Families and Genera of the Bats." This work is scarcely as adequate as his catalogue of the European Mammals, inasmuch as his material was scantier, but in spite of this it is work of the very highest quality. We obtain a curve (Fig. 3-D) of almost as aberrant a nature as the curve based upon MacGillivray's "The Coccidae," which work stands in striking contrast, so far as quality is concerned, to that of Miller. Assuming the validity of Miller's data, the most obvious explanation of the great preponderance of monotypes as coupled with the numbers of larger genera is that here we are dealing with a relatively little known group. This is in accordance with what we know from other sources to be the case. Miller himself estimates that perhaps 60 per cent. of the group is known at present.

Finally, in this connection, are we safe in assuming that complete and accurate systematic data will give this hollow curve? I am personally convinced that it will. But should such a result be recognized in our nomenclature? At present I feel inclined to say no, with some qualifications. On the other hand, I feel much less positive on this point than when I first considered it. While recognizing the smallness of the numbers involved, it may be instructive to consider the following example.

Thus, having recently completed a monograph of the Tachardiinae or lac insects, I was interested in determin-

ing the form of the curve given by plotting the data here derived. There were 44 species recognized, which, on the basis of our theoretical curve, should give us six or seven monotypes, three or four ditypes and finally a single genus of six or seven species. As a matter of fact I had recognized nomenclatorially only four genera and two subgenera, which plotted give a perfectly straight line. On the other hand, I recognized and segregated groups and subgroups which, if considered as genera and plotted as usual, give a curve which practically coincides with its ideal (Fig. 2-A). Now then the question is this. Was I right or wrong in not recognizing all these distinct groups and subgroups as valid genera and should I have recognized them nomenclatorially? Certainly the characters used in separating them are no less constant and definite than those used by some systematists in distinguishing their genera. In other words, does the straight line lying wholly beneath its ideal curve given by my *named* genera and subgenera indicate an ultra-conservative classification, *nomenclatorially* speaking? At present I am not willing definitely to commit myself one way or another, but I should certainly be somewhat hesitant about proposing a series of new names for the groups and subgroups involved in this discussion, a procedure which without doubt would be followed by some workers. And which of these procedures is logically correct? It seems to me that if we may ever hope to attain a very close approximation to a natural system of classification we will have to devise some means for handling and distinguishing groups and sub- and supergroups whether or not we recognize them by names. The preceding discussion shows plainly that our hollow curve gives us an entirely new standpoint from which to rationally consider the "genus."

As an illustrative example purely, there is given the curve of the Coniferae (Fig. 3-E) as obtained by plotting the data given by Willis in his "Dictionary of the Flowering Plants and Ferns." It shows very beautifully the wide separation of the curve from its ideal and again

serves to emphasize the necessity of a standard of comparison such as our ideal curve, for a real appreciation of the peculiarities of individual curves. The curious irregularities in this curve seem to primarily call for a reinvestigation of the data involved. Assuming its validity, a point upon which I can voice no opinion, it seems to indicate that some very complex evolutionary factors have been at work modifying the generic ratios. Certainly more than senescence or phyletic differentiation is at work.

As a final interesting case, and one which opens up a number of interesting possibilities, is the curve for the flora of the Pollard Willow trees near Cambridge, England, as determined and reported upon by Willis and Yule. In this case species are plotted against the frequency of their occurrence in these willow trees. The willow tops, being in fact a virgin territory, may be considered as a new area being populated by an overflow of species from surrounding regions. Of course this is not strictly true, inasmuch as each willow top probably derived its flora independently from the surrounding territory instead of from the adjacent willows. However, as the curve shows (Fig. 3-F) we have here an ideal illustration for showing definitely that an overflow flora will independently give the hollow curve so far as age and area is concerned. As Willis stresses, a lucky accident may have placed a plant with a very poor dispersal apparatus among the first arrivals in these willows, but on the whole there is little doubt that the poorer the dispersal apparatus, the later on the average would the plant concerned gain a foothold in these places. And there is scarcely room for doubt that if the whole history were known, the species with the fewest records would represent the latest arrivals. The curve does indeed lie considerably below the ideal, but nevertheless it is remarkably similar in general form to typical examples.

I have not considered the correlation between age and area so closely, but what study I have given it clearly in-

dicates that the curve derived by plotting generic size to area lies definitely higher and beyond the corresponding size to frequency curve. This may be demonstrated theoretically by calculating its ideal form as was previously done for the generic size to frequency curve. It also works out very clearly in practice, but this data I am reserving for a future treatment.

Thus we assume the same type of generic and specific increase to hold as before outlined and in addition assume that species will at their inception occupy a circular area, whose radius may for simplicity be taken as equal to unity. Then assume that during each phylogenetic time interval, our species will spread radially a distance equal to unity. This gives our ordinary dichotomous progression modified by the " πr^2 " factor involved in determining the area of our theoretically circular range. As is shown in Fig. 1-A this is a considerably more rapid rate of increase than the ordinary dichotomous progression. Then, calculating our results at any finite point, here for the sake of convenience again taken at 64, we obtain the data graphically expressed in the curve shown in Fig. 1-C. As may be seen at a glance it lies entirely distinct from and beyond the corresponding curve for the phylogeny of our hypothetical group. In short, the rate of distribution in space (geographical distribution) is distinctly greater than in time (paleontological distribution).

As before mentioned, actual data, in many cases at least, conform very closely to the above derived hypothetical curve. In dealing with actual data it is of course obvious that extremely few cases of even approximately circular ranges will appear. This deficiency may in large part be obviated, especially in comparatively young groups, by taking the outlying stations of a given genus or species and calculating therefrom a theoretical center of dispersal and theoretical radius. This is what has been done in obtaining the results here referred to.

Before concluding it might be worth while to call atten-

tion to a large class of phenomena which give curves of striking similitude to the true hollow curve. Thus data giving an extremely skewed frequency distribution will fall into this class. For example, the wealth curve shows this resemblance perfectly. To illustrate this point there is given the curve derived by plotting the frequency with which incorporated cities of certain sizes appear in the state of California. Fig. 3-G. As here shown, the comparatively few incorporated towns of less than 1,000 inhabitants are lumped with the thousand class and as a result we obtain a beautiful and typical hollow curve. Actually we should have plotted these cities in their proper position and in that case the peak of the curve would have been slightly lowered and the curve drawn sharply downward to the base line at about the 300 population, size limit—giving us a splendid example of a sharply skewed curve.

It may be argued, then, that all these so-called hollow curves are nothing more than extreme skew forms. This position is not believed to be tenable, however, inasmuch as the nature of the data involved does not permit us to consider sizes nor frequencies of less than unity. A fractional genus or species has no valid existence.

To recur to one of the most interesting problems involved in this study, that is, as to whether we “should” obtain the hollow curve, granting for the moment at least the soundness of the theory of regular dichotomous generic increase, in view of the innumerable disturbing ecological factors. Personally, I believe it should. As a matter of fact, there is required just some such basic theory of as fundamental a nature as this to furnish an adequate reason for the curves’ actual occurrence. And finally, while freely admitting the lack of positive proof for this underlying, theoretically ideal method of growth, nevertheless, the substantiation offered by the curve itself, as well as by the fact that we find this simple type of increase common to organic reproduction in general (may it not be a direct consequence of organic reproduc-

tion in general?), makes its use seem perfectly logical and safe as a working hypothesis. Its very simplicity is an argument in its favor. Naturally, we can not expect it to work out with the fluency of a mathematical progression, but at any rate this should not vitally impair its usefulness in practice. Modifying and "upsetting" factors are no more than the "impurities in our reaction." They correspond exactly to the contaminations and counter-reactions to be found in chemical combinations as they normally occur in nature.

In conclusion I should like to call attention to the vital need of more complete and accurate systematic work. What we need first of all, before we can proceed with any assurance, to prove or disprove such theories as have been advanced in this paper, is adequate and accurate data. It is no more than axiomatic to reiterate that results can be no better than the data upon which they are based (and they may be much worse), and it is only too obvious that much of the enormous mass of systematic work already done, does *not* conform to these requirements.

As in all other branches of science, accurate observation and data gathering must precede the formation of accurate generalities. A Kepler must precede a Newton. And here where we have complicating factors assuming such a tremendous rôle when coupled with the complexity of biotic phenomena in general, the need is obviously even greater. It is not too much to say that the gathering of accurate systematic data calls not only for the very highest type of individual observational ability, but also for the highest form of collaborative and correlative work. And in referring to systematic data, I mean it in its broadest sense. The careful and critical collection and organization of every scrap of evidence, from whatever the source, having a possible bearing on the phyletic affiliations of a group of organisms, constitutes in my mind true systematic work. And correlated with, and a vitally necessary part of this work, is the "bookkeeping"

or taxonomic nomenclatural handling of the group. After all, it is as necessary that the biologist be able to deal with species of known characteristics, as it is that the chemist be able to deal with chemically pure substances, and the name of an organism, insofar as it is accurately determined by a competent man, is the "trademark and guarantee" of our material. This point is only brought up here because it is only too apparent that many biologists, if not openly contemptuous of the taxonomist or, as I prefer to call him, the systematist, seem to forget that *his* is the tie that binds, in large part at least, whatever information their own researches have yielded into the general fabric of our biological knowledge.

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SHORTER ARTICLES AND DISCUSSION

A KEY TO THE EGGS OF THE SALIENTIA EAST OF THE MISSISSIPPI RIVER¹

FOR the last two years the authors have been intensively studying the life-histories of the Salientia (frogs, toads, tree-frogs, etc.) of the Okefinokee Swamp, Georgia. A summary of one phase of the subject, namely, eggs, is herewith presented. Incorporated with this consideration of our southeastern species is a previous key to the eggs of our northeastern forms. The combination may be termed "A key to the eggs of the Salientia east of the Mississippi River."

Workers will discover the following species missing from the key because the authors themselves have had no opportunity to secure both fresh and preserved materials of these species:

Hyla andersonii—Eggs first found by T. Barbour and later by G. K. and R. C. Noble.

Hyla evittata—Eggs not known. Doubtless like *Hyla cinerea*.

Pseudacris ornata—Eggs not known.

Pseudacris nigrita—Eggs most certainly like *Pseudacris triseriata*, *P. feriarum*, and *P. septentrionalis*.

Rana arcolata—Eggs not known, probably of *R. pipiens* type.

Rana cantabrigensis—Eggs very similar to *R. sylvatica*.

Rana septentrionalis—Eggs described by Garnier. After this paper went to press the authors and Mr. S. C. Bishop discovered eggs of this species. The description is: Egg mass submerged, a plinth; 1.5×3 inches to 3×6 inches; vitellus black or brownish above, white or yellowish below; vitellus, average 1.4 mm; range 1.3–1.6 mm; inner envelope, average 2.7 mm; range 2.4–3 mm; outer envelope, average 6.3 mm; range 5.6–6.6 mm. Season, June 25–July 25.

The season of breeding for species in the north is marked both at the beginning and at the end. Each species occupies four or five weeks except *Bufo americanus* and *Rana clamitans*. The exceptions may require two or three months for ovulation. In

¹ The investigation upon which this article is based was supported by a grant from the Heckscher Foundation for the Advancement of Research, established at Cornell University by August Heckscher.

In the summer of 1921 Mr. Francis Harper assisted in the field work of this study and in the summer of 1922 both he and Mr. Miles D. Pirnie gave valuable assistance to the authors in this same series of studies.

the southeastern states when once a species has begun, its season of breeding may extend throughout the summer or even into the early fall, dependent upon the high crests of precipitation. These species, although of a swampy region, wait for the rains

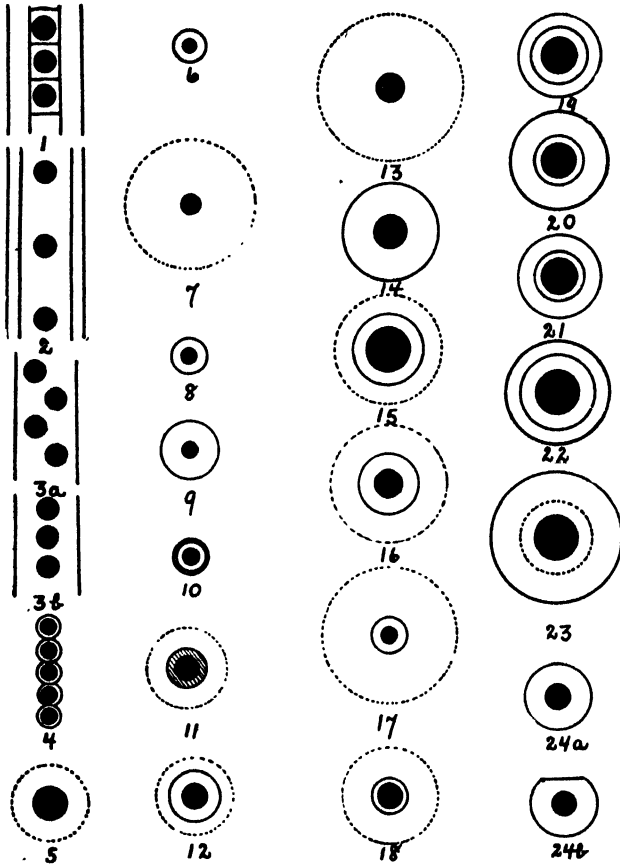


PLATE I. INDIVIDUAL EGGS. $\times 2.3$

Fig. 1. *Bufo americanus*.

2. *Bufo terrestris*.

3. *Bufo fowleri*.

4. *Bufo quercicus*.

5. *Scaphiopus holbrookii*.

6. *Pseudacris ocularis*.

7. *Pseudacris triseriata*.

(From Buffalo, N. Y.).

8. *Hyla crucifer*.

9. *Acris gryllus*.

10. *Hyla squirella*.

11. *Hyla gratiosa*.

12. *Hyla cinerea*.

Fig. 13. *Rana catesbeiana*.

14. *Rana virgatipes*.

15. *Rana grylio*.

16. *Rana clamitans*.

17. *Hyla femoralis*.

18. *Hyla versicolor*.

19. *Rana palustris*.

20. *Rana pipiens*.

21. *Rana sphenoccephala*.

22. *Rana aesopus*.

23. *Rana sylvatica*.

24. *Gastrophryne carolinensis*.

In Fig. 15 the vitellus was inadvertently drawn too large.

and in this reliance on precipitation they suggest our desert species of Texas and Arizona. Those which do not begin until June have at least eight to ten weeks of ovulation. This minimum period for a species of the south is the maximum period for a northern form. Species such as *Rana sphenoccephala*, *Bufo terrestris* and *Acris gryllus*, which begin early in the season, breed during 25-30 weeks of the year, if not longer or from February to September or October.

The number of eggs in a complement may vary from 100 in the smallest species, *Pseudacris ocularis*, to 20,000 in *Rana catesbeiana*, the largest form. The range in the tree-frogs (*Hylidae*) is from 100 (*Pseudacris ocularis*) to 2,084 (*Hyla gratiosa*); in the toads (*Bufonidae*) from 610 (*Bufo quercicus*) to 8,000 (*Bufo americanus*); in the frogs (*Ranidae*) from 349 (*Rana virgatipes*) to 20,000 (*Rana catesbeiana*). The complements of the narrow-mouthed frog (*Gastrophryne carolinensis*) and the spadefoot (*Scaphiopus holbrookii*) are, respectively, 869 and 2,332.

The eggs of seven species, *Hyla cinerea*, *H. femoralis*, *H. versicolor*, *Rana catesbeiana*, *R. clamitans*, *R. gryllio* and *Gastrophryne carolinensis* float on the surface of the water; the eggs of the other 17 species are submerged. In northern or southeastern states no form with buoyant eggs lays before May 10. The 11 or 12 early breeders have submerged eggs. These are usually with firm jelly envelopes except for *Pseudacris triseriata* and *Scaphiopus holbrookii*, which have the consistency intermediate between the firm jellies of early breeders and the loose surface films of late breeders. One form, *Gastrophryne carolinensis*, although it lays at the surface, has the most beautifully distinct, firm eggs of all the species considered.

The eggs were laid in camp and in the laboratory by mated pairs caught in the field. Later the eggs in the field were determined by these original checks. Occasionally the process of egg laying was observed in the field. Two species, one of the north and one of the south, were identified by the positive elimination of all the other resident forms.

The measurements and color descriptions are based on fresh eggs. Later, these were checked with preserved material. The eggs with loose outer envelope have the outer margin indicated by dots. In one species the vitelline membrane is far separated from the vitellus and the space is indicated by cross hatching. A summary of the egg characters of each species follows in the accompanying key:

A. Eggs deposited singly.

a. Envelopes two.

Outer envelope diameter 1.4 to 2.0 mm; inner envelope diameter 1.2 to 1.6 mm; vitellus diameter 0.8 to 1.0 mm; eggs brown above and cream below. Egg-complement, 942. Season, June 10 to August 21 (Fig. 10).....*Hyla squirella*

aa. Envelope single.

b. Envelope 2.3 mm or more.

c. Vitelline membrane far from vitellus, appearing as inner envelope 1.6 to 2.0 mm; outer envelope loose, glutinous, indefinite in outline 2.3 to 5 mm; vitellus 1.0 to 1.8 mm. Egg-complement, 2084. Season, June 10 to August 21 (Fig. 11).....*Hyla gratiosa*

cc. No inner envelope or appearance as such, envelope firm, definite in outline 2.4 to 3.6 mm; vitellus 0.9 to 1.0 mm. Egg-complement, 241. Season April 15 or earlier to September 1 (Fig. 9).....*Acris gryllus*

bb. Envelope 1.2 to 2.0 mm.

c. Vitellus 0.6 to 0.8 mm. Egg-complement, 100. Season, May 16 to August 21 (Fig. 6).....*Pseudacris ocularis*

cc. Vitellus 0.9 to 1.1 mm. Egg-complement, 800 to 1,000. Season, March 30 to May 15 (Fig. 8).....*Hyla crucifer*

AA. Eggs deposited in a mass.

a. Egg mass, a surface film.

b. Egg envelope outline always distinct, never lost in the mass; eggs firm and distinct like glass marbles, making a fine mosaic; envelope a truncated sphere, the flat surface above; envelope single 2.8 to 4.0 mm; vitellus 1.0 to 1.2 mm; color black above and white below. Egg-complement, 869. Season, May 21 to August 17 (Fig. 24).....*Gastrophryne carolinensis*

bb. Egg envelope outline indistinct, more or less merged in the jelly mass; jelly glutinous; egg brown above, cream or yellow below.

c. Egg packets small, masses seldom if ever over 20 sq. in. (125 square centimeters), or 4 by 5 inches in diameter (10 by 12.5 cm).

d. Inner envelope large 2.2 to 3.4 mm; outer envelope 3.2 to 5.0 mm; vitellus 0.8 to 1.6 mm. Egg-complement, 343 to 500. Season, May 19 to August 21 (Fig. 12).....*Hyla cinerea*

dd. Inner envelope small 1.4 to 2.0 mm; outer envelope 4 to 8 mm.

e. Packets small, seldom over 30 to 40 eggs; vitellus 1.1 to 1.2 mm. Egg-complement, 1,802. Season, May 10 to August 13 (Fig. 18).....*Hyla versicolor*

ee. Packets large, sometimes 100 to 125 eggs; vitellus 0.8 to 1.2 mm, av. 0.95 mm. Egg-complement, 768. Season May 16 to August 21 (Fig. 17)

.....*Hyla femoralis*

- cc. Egg packets large, loose, glutinous films, 35 sq. in. to 675 sq. in. (218 to 3,721 sq. cm).
- d. Inner envelope absent; vitellus 1.2 to 1.7 mm; egg mass 144 to 675 sq. in. (900 to 3,721 sq. cm) in area, or 12 by 25 inches (30 by 61 cm) in diameter; egg masses amongst brush around the edge of ponds or encircling *Pontederia*-like vegetation in mid pond. Egg-complement, 10,000 to 20,000. Season, June 1 to July 10 (Fig. 13). *Rana catesbeiana*
- dd. Inner envelope present, 2.8 to 4.0 mm; vitellus 1.4 to 2.0 mm.
- e. Egg mass seldom 1 sq. ft. (35 to 144 sq. in. or 218 to 900 cm) in area or 5 by 7 to 12 inches in diameter; usually around edge of ponds; inner envelope elliptic, pyriform or circular, av. 3.05 mm; vitellus 1.4 to 1.8 mm, mode 1.4 mm, av. 1.5 mm. Egg-complement, 1,451 to 4,000. Season, May 23 to August 21 (Fig. 16) *Rana clamitans*
- ee. Egg mass over 1 sq. ft. in area (144 to 288 sq. in. or 900 to 1,800 sq. cm) or 12 by 12 inches to 12 by 25 inches in diameter; usually in midpond; inner envelope av. 3.45 mm; vitellus 1.4 to 2.0 mm, mode 1.8 mm, av. 1.7 mm. Egg-complement, 8,000 to 15,000. Season, May 24 to August 21 (Fig. 15). *Rana grylio*
- aa. Egg mass submerged.
- b. Eggs in files or bands.
- c. Eggs laid in bands which soon become loose cylinders extending along plant stems or grass blades; vitellus 1.4 to 2.0 mm; envelope 3.8 to 5.6 mm. Egg-complement, 2,332. Season, April 15 or earlier to August 17 (Fig. 5) *Scaphiopus holbrookii*
- cc. Eggs in files.
- d. Files short (4 to 10 mm in length); 4 to 8 eggs in short bead-like chain or bar or many such files radiating from one focus; vitellus 0.8 to 1.0 mm; tube diameter 1.2 to 1.4 mm. Egg-complement, 610, 766. Season, June 4 to August 21 (Fig. 4). *Bufo quercicus*
- dd. Files long (several feet in length or often a meter or more long); vitellus 1.0 to 1.4 mm; tube diameter 2.6 to 4.6 mm.
- e. Inner tube absent; outer tube 2.8 to 3.4 mm; vitelli crowded in the files; at first in a double row, later more spread out but still crowded; 22 to 24 eggs in 30 mm (1 3/16 inches). Egg-complement, 7,750. Season, April 15 or earlier to August 17 (Fig. 3) *Bufo fowleri*
- ee. Inner tube present.
- f. 18 to 20 eggs in 30 mm (1 3/16 inches); partition apparent between eggs; inner tube 1.6

- to 2.2 mm; outer tube 3.4 to 4.0 mm. Egg-complement, 4,000 to 8,000. Season, April 5 to July 25 (Fig. 1).....*Bufo americanus*
- ff. 7 to 8 eggs in 30 mm (1 3/16 inches; distinct space between eggs; no partition apparent; tube inclined to be slightly emarginate between the eggs; inner tube, 2.2 to 3.4 mm; outer tube, 2.6 to 4.6 mm. Egg-complement, 2,888. Season, April 15 or earlier to August 13 (Fig. 2). *Bufo terrestris*
- bb. Eggs in lumps.
- c. Egg mass a firm regular cluster.
- d. Egg mass a sphere 2½ to 4 in. (6.35 to 10 cm) in diameter, containing 2,000 to 3,000 eggs; outer envelope distinct.
- e. Eggs black above and white below; inner envelope apparently absent, slightly evident under lens, 3.6 to 5.8 mm; outer envelope 5.2 to 9.4 mm; vitellus 1.8 to 2.4 mm. Egg-complement, 2,000 to 3,000. Season, March 19 to May 1 (Fig. 23)
.....*Rana sylvatica*
- ee. Eggs brown above and yellow below; inner envelope distinct 2.3 to 3.0 mm; outer envelope 3.6 to 5.0 mm; vitellus 1.6 to 1.9 mm. Egg-complement, 2,000 to 3,000. Season, April 6 to May 18 (Fig. 19) *Rana palustris*
- dd. Egg mass a plinth.
- e. Without inner envelope, complement small, 349 to 474; eggs black above and sulphur or primrose yellow below; eggs further apart than in *R. pipiens* or *R. sphenoccephala*; outer envelope 4.9 to 6.9 mm; vitellus 1.5 to 1.8 mm. Egg-complement, 349 to 474. Season, June 21 to August 11 (Fig. 14)
.....*Rana virgatipes*
- ee. With inner envelope, complement large, 1,000 to 5,000 or more; eggs black above and white below.
- f. Vitellus average 2.0 mm (range 1.8 to 2.4 mm); inner envelope 3.1 to 4.4 mm; outer envelope 4.4 to 6.0 mm, mode 5.2 mm, av. 5.3 mm. Egg-complement, 5,000 or more. Season, (†) to August 17 (Fig. 22).*Rana aescopus*
- ff. Vitellus average 1.7 mm (range 1.4 to 2.0 mm); inner envelope 2.3 to 3.2 mm; outer envelope 3.4 to 6.0 mm. Egg-complement, 1,000 to 5,000.
- g. Average outer envelope 5.1 mm (range 4.2 to 6.0 mm, mode 5.0 mm). Egg-complement, 3,500 to 4,500. Season, March 30 to May 15 (Fig. 20).....*Rana pipiens*

gg. Average outer envelope 3.8 mm (range 3.4 to 5.4 mm, mode 4.0 mm). Egg-complement, 1,054. Season, April 15 or earlier to August 21 (Fig. 21)

... .. *Rana sphenocephala*

cc. Egg mass a loose irregular cluster.

- d. Egg mass small, less than 1 in. (2.5 cm) in diameter. 20 to 100 eggs in the mass; outer envelopes merged; the one envelope 5.0 to 7.8 mm, rarely 3.0 mm; vitellus 0.9 to 1.2 mm. Egg-complement, 500 to 800. Season, March 19 to May 1 (Fig. 7).. . *Pseudacris triseriata*
- dd. Egg mass an irregular cylinder 1 to 6 in. (2.5 to 15 cm) in length, extending along plant stem or grass blade; envelope, single, 3.8 to 5.6 mm; vitellus 1.4 to 2.0 mm. Egg-complement, 2,332. Season, April 15 or earlier to August 17 (Fig. 5). . *Scaphiopus holbrookii* (See also (c) under (b) Eggs in files or bands.)

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SOME CHROMOSOME NUMBERS IN NICOTIANA

FOR some years certain genetic experiments involving numerous species and varieties of the genus *Nicotiana* have been carried on by the Department of Botany of the University of California¹. In connection with a number of these investigations cytological information has for some time been greatly needed. The writer has had the privilege, under a fellowship grant from the Scandinavian-American Foundation, of spending some months in the laboratory of Professor Otto Rosenberg and of there making some start in securing such information. The present note deals with chromosome numbers of certain more familiar species of *Nicotiana*. The determination of chromosome numbers was preliminary to work upon the cytological phenomena exhibited by hybrids between *N. sylvestris* and varieties of *N. Tabacum*, concerning which another note is in preparation.

The chromosome number of but one species of *Nicotiana* has as yet been reported in the literature. White² and Palm³ have

¹ The results of these experiments have been published in part in the Univ. Calif. Publ. Botany, Vol. 5, Nos. 1-17.

² White, O. E.—AMER. NAT., Vol. 47, p. 206, 1913.

³ Palm, B. T.—Bull. Deli Proefstat. Medan, No. 16, 1922.

found x-24, while Arisz⁴ reports x-25 for *N. Tabacum*. The following list is derived from studies of fixed material made by the writer and confirmed in most cases* during the summer of 1922 in California, by Dr. Margaret Mann according to Belling's aceto-carmin method.

x— 9: *Langsdorffii** (2 vars.), *alata* (*affinis*)*, *longiflora*;

x—12: *sylvestris**, *glauca**, *suaveolens**, *glutinosa**, *paniculata**, *acuminata*;

x— 24: *Tabacum** (5 vars.), *rustica**, (3 vars.), *Bigelovii**, *nudicaulis*.⁵

Three of these counts are open to some question. In the case of *N. alata* homotypic anaphase plates in the fixed material show 10 chromosomes in some cases, although the predominating number in such stages is 9. Similarly, *N. longiflora* can be counted as 9 or 10, but in this case the predominating number is 10. Only a small amount of fixed material of *N. suaveolens* was available and there is some doubt as to whether the number is not larger than 12, possibly 18. Petaloid anthers of *N. Tabacum* var. *calycina* showed numbers of abnormal heterotypic metaphases, involving apparently the presence of both univalent and bivalent chromosomes. Rather striking differences in chromosome size were observed among the various species but in the absence of controlled, duplicate fixations little significance can be given to this evidence.

Further examination of the species the chromosome numbers of which are here reported will be made this summer when material of a number of other species should be available. An effort is being made to grow a still larger collection of *Nicotiana* species for similar examination in the hope that a combination of genetic and cytological evidence may give a basis for a more rational taxonomic treatment of the genus than has as yet been offered.

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APRIL 17, 1923

⁴ Quoted from Palm, p. 16.

⁵ For descriptions of the plants grown under these species designations see Setchell, W. A., Univ. Calif. Publ. Botany, Vol. 5, No. 1, 1912.

A REPORT OF A HISTOLOGICAL STUDY OF THE EYES
AND GONADS OF MICE TREATED WITH A
LIGHT DOSAGE OF X-RAYS

(1) INTRODUCTION

THE object of this experiment is to determine whether exposure to a light dosage of X-rays over the whole body has any direct effect upon the histological structure of the eyes and of the gonads in adult mice.

Because of the appearance of genetic modifications among the descendants of mice given a similar dose to that described below (Little and Bagg and Bagg and Little now in press) it was thought to be of interest to determine whether or not there was, in animals of the same parent stock, a direct effect of the X-rays of sufficient magnitude to produce histological modifications.

The eyes were chosen for particular study because at least two of the genetic modifications above referred to expressed themselves in the eye, and because Stockard, Guyer and Smith, and others have found that the eye is a common seat for variation under experimental conditions.

The gonads were chosen because in the case of the female, and probably of the male as well, it is certain that the genetic modifications which appeared were at their origin situated in the germ cells. If they are, then, a direct result of or accompanied by gross histological changes, that fact should be easy to recognize.

(2) MATERIAL AND METHODS

A total of 60 adult mice were used, consisting of 17 black males, 17 black females, 11 brown males and 15 brown females. Three of each of these four groups were killed every three days. The exposures to X-rays were made at the Memorial Hospital in New York City, by the courtesy of Dr. H. J. Bagg. Twelve mice placed in a small wire cage were subjected at one time to the X-rays. No filter was used. The distance was 13 inches, and the strength was ten milliamperes, while the time was 12 seconds. This was done each day for five successive days.

Beginning two days after the last treatment, three mice from each group were killed each day, and the eyes and gonads were removed. The eyes were fixed in Zenker's fluid, and stained with

Delafield's hematoxylin and eosin. The lenses were removed after fixation. The gonads were fixed in Bouin's solution and stained with Delafield's hematoxylin and eosin. These preparations were compared with eyes and gonads of unexposed animals similarly prepared.

(3) RESULTS

In the gonads of the X-rayed animals, no changes were observed. In the testes even after the fourteenth day after treatment, active spermatogenesis was found. No degeneration or gross change of any kind could be noted. Likewise in the ovaries eggs were developing normally. No difference was observed in the eyes of the treated and untreated animals.

(4) DISCUSSION AND CONCLUSION

It will thus be seen that no direct effect of the X-ray exposure given to the ancestors of the abnormal individuals reported by Little and Bagg was observed. The possibility of the inheritance of an acquired character or characters being involved in this case is therefore apparently done away with and the whole matter falls much more properly under the head of a direct effect of the germ plasm by the rays themselves.

Of course the possibility that there was on the eye itself a direct effect of a very minute nature still exists, but it seems both from the absence of visible effects and from the regularity of the process of inheritance as described by the investigators referred to, that each genetic modification is the result of a minute and probably direct change in a very limited region of the chromatin material or even in a single gene.

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HUMAN INHERITANCE¹

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STUDENTS of heredity, having found abundant evidence that the same laws of inheritance apply to plants and animals, both wild and domesticated, could not help but look at their own species with curious eyes. It was obvious that mankind shows great variability within each race—as great as that of cultivated plants and domesticated animals, and it was soon discovered that some at least of the variations conform to familiar types of Mendelian heredity.

Many of the characters, shown by our domestic animals and plants, probably arose in prehistoric times, but the origin of many others is known. In general, it may be said that some of these characters have been obtained by crossing related wild varieties to each other, while others have arisen independently of crossing. The great majority of the latter, perhaps all of them, arise as sudden and random changes in a localized region of the germ-material by mutation, or, as we say, by a change in a gene. I shall consider later whether some of the characters that are immediately concerned with the welfare of the animal—its adaptive characters—may or may not have had a still different origin.

Since we can not be certain which characters in our domesticated forms, and in man, have arisen through crossing and which have arisen by mutation, it may help

¹ Ninth Mellon Lecture, delivered May 7, 1924, at Pittsburgh, Pennsylvania, under the auspices of the Society for Biological Research of the School of Medicine, University of Pittsburgh.

the later discussion if I first describe some of the mutant types in one species with which I am most familiar. For twelve years the origin of variations in the vinegar fly, *Drosophila melanogaster*, has been observed. Over 400 new characters have appeared. Since these characters are not present in the wild species, and since this fly crosses with only one other species, and then gives sterile hybrids, it can not be said that the new characters originated from crossing to other species. Here, then, we have an opportunity to see what kinds of changes may arise by mutation in single genes and how they are inherited. From this standpoint we may then proceed to compare the results with what we find in domesticated forms and in man himself.

The new mutant characters of *Drosophila* suddenly appear sometimes in single individuals, sometimes in a few individuals, and sometimes (from pair matings) in a quarter of the offspring. When single individuals appear they are generally males,² or else the characters are dominant; when in larger numbers the individuals do not represent the initial mutation that produced them, but arise from the germ-cells of two individuals, each of which has received from a common ancestor the recessive genes in question.

The numerical results indicate that a new character is traceable in all cases to a change that has taken place in one of the chromosomes in the germ-track. This is a very

² Since the son gets his single X-chromosome from his mother he will at once show any recessive character carried by that chromosome. Therefore, if a mutation had occurred in one of the maternal chromosomes and had the egg that carried the chromosome after maturation been fertilized by a Y-bearing sperm (that carries no dominant factors) the mutant gene would produce its effect. On the other hand, had this egg been fertilized by an X-bearing sperm (whose X carried the normal allelomorph of the postulated new gene) the presence of the mutant gene would not be seen in the resulting heterozygous daughters; but when they, in turn, produced offspring, half of the males would get the chromosome in question and show the new character. If the recessive mutation were not in the X-chromosome but in another one, then it could come to expression only when a male and a female, each carrying one such chromosome, mated. One quarter of the offspring would then exhibit the mutant character.

interesting feature of the mutation process, and one that we have watched with absorbing interest in *Drosophila* for several years. When we recall that in each cell, including those of the germ-track, there are two chromosomes that are identical in the kind of hereditary units that they carry, it is extraordinary that the mutant change should take place in only one of the two members of the chromosome pair.³ It is difficult to imagine how any outside influence could have a specific effect on one spot of one chromosome without affecting the same change in the identical spot in the corresponding chromosome. We know, however, too little of the nature of the chemical or physical event involved when a mutation occurs to make it worth while at present to do more than record the facts.

We have no reason for supposing that these mutations are due to confinement or to any of the special conditions connected with confinement. On the contrary, there is evidence indicating that in wild species the same kinds of mutations are always appearing. It must be supposed, therefore, that the germ material is by no means completely stable, or rather that it is quite unstable, as we would anticipate for such a complicated organic structure as protoplasm.

The 400 characters of *Drosophila* fall into four linkage groups. I mean by this that if certain characters enter together from one parent they tend to stay together in subsequent generations—not absolutely, it is true, but according to definite laws. We explain this on the theory that characters are linked when their genes lie in the same chromosome. The chromosomes are supposed to be continuous from one cell-generation to the next. There are four pairs of chromosomes in *Drosophila*, and there is

³ The case of the reversion in the eye character, Bar, recently reported on by Sturtevant and Morgan, seems to be a unique process of mutation, since it occurs only by a crossing-over between the two X-chromosomes of the female. Whether some other mutations may also be due to a similar process is not known, but since sex-linked mutations also occur in the male *Drosophila* (that has but one X) there is no reason to suppose that all mutations owe their origin to crossing-over.

both genetic and cytological evidence that each linked series of genes is carried by one of these four kinds of chromosomes. Since there are two chromosomes of each kind (except in the male that has an XY pair) there are two linked sets of genes corresponding to each of the four chromosome pairs.

There are many kinds of characters in each linkage group. The first group consists of characters carried by the X-chromosomes. These characters include changes in the color of the eyes, changes in the wings or legs or bristles of the body or the general color of the whole fly. Since there are two X-chromosomes in the females that carry the genes of these characters, and only one X in the male, the inheritance of these sex-linked characters is different from that of the characters of the other linkage groups. The mate of the X-chromosome in the male, the Y-chromosome, is, in a sense, empty, or at least it carries no genes like those in the other chromosomes. In *Drosophila*, its presence may be ignored, but the X and the Y of the male form a pair when the chromosomes conjugate. They separate when the sperm matures, and half of the spermatozoa carry the X and half the Y. When the XX egg matures, one X is left in each egg. If an egg is fertilized by an X-bearing sperm a female is produced. Hence, half the offspring are females and half males. The mechanism gives an equality of the sexes.

This method of sex regulation is not peculiar to *Drosophila*, but is present in many of the insects, and is found in several other great groups of animals. In man, also, with 48 chromosomes according to Painter, the male is XY, and the female XX. It is significant, therefore, to find in man several characters that are inherited in the same way as are the sex-linked characters in *Drosophila*.

The second linkage group of *Drosophila* also contains genes that affect all parts of the animal, the color of the eyes, the color of the whole animal, the shape of the wings or of the legs or of the hairs. The third linkage group is equally large, and we find again changes in all parts

of the body as before. The fourth linkage group is a small one. At present only four characters have been discovered that belong to this group. It has been definitely proved that they are carried by the very small fourth pair of chromosomes.

We have been able to trace these characters not only to specific chromosomes, but to determine where the genes lie within each chromosome. The method of locating the genes I need not describe here, but it is important to note that the order of the genes bears no relation to the arrangement of the parts of the body, where they produce their most striking effects.

These mutant characters are inherited, individually, according to Mendel's laws and the extension of those laws that have more recently been discovered. The great majority of the characters are recessive, a few are dominant. This distinction is largely arbitrary and apparently has no real importance. In fact, only by courtesy can many characters be said to be dominant or recessive, since each gives with the wild type an intermediate character which means that both genes have an influence on the result. The really important fact connected with this question is the separation of the members of each pair of genes at the time of maturation of the germ-cells. It has been shown beyond any question that this separation (segregation) takes place in exactly the same way in a hybrid whose character is intermediate as in a hybrid that shows more completely the dominance or the recessiveness of a character.

In most cases a single kind of mutation occurs in the same spot (locus) of the chromosome, but as many as ten different modifications of the so-called white eye locus of *Drosophila* have been recorded, and several other cases of the sort, where multiple allelomorphs have appeared, are well known.

There are certain mutations that express themselves only when some other modification is present. These are spoken of as specific modifiers. For example, there is a

recessive gene, called cream, that produces an effect when another mutant character, eosin eye color, is present, but it produces no visible effect on the red eye color of the normal eye. These specific modifiers do not, however, differ in any essential respect from the double effect shown by two genes both of which affect the same character. Thus the eye color called vermilion is a bright red eye. Eosin is a pinkish yellow eye color. The double recessive eosin vermilion is almost white. Vermilion might be said to be a modifier of eosin or eosin might be said to be a modifier of vermilion. In principle this is not different from the effect of cream on eosin, or of eosin on cream, except in so far as cream alone does not produce any effect on the wild-type eye color. In general it may be said that modifying factors play an important rôle in producing small differences in characters—differences that are inherited. So slight are some of these effects that the variations produced may not be greater or even as great as those fluctuating differences that are due to the environment. It often calls for the most refined genetic methods to demonstrate whether or not slight differences are due to genetic modifying factors or to the environment. It is owing to the difficulty of distinguishing between these two kinds of effects that so much of the earlier work on selection fell into serious error.

It is here that the modern study of genetics comes into closest contact with the theory of evolution through natural selection. This theory postulates that the small differences that are shown by different individuals of a species furnish the materials on which selection acts. To-day we realize that many of these individual differences are due to environmental influences affecting the developmental stages, and that they are not inherited, but we also realize that many of the individual differences are due to the presence of genetic modifying factors that affect particular parts, making them more (or less) extreme than they are in the average individual. Should it be an advantage to have a particular part more devel-

oped it will be favored by selection, and when this difference is due to a genetic modifying factor the modification will be inherited. The discovery that many of these quantitative relations are due to modifying genes that are inherited in the same way as are the major effects shown by extreme mutants is, as I have said, the special contribution of genetics to the theory of evolution. Perhaps the most significant fact that a study of the mutant genes of *Drosophila* has brought to light relates to the manifold effects produced by each gene. Since we name the character from the most striking effect of the gene, which receives the same name, the impression is produced that each gene affects only a particular part of the body in a particular way. We are apt to forget that the evidence shows that, as a rule, many other parts of the body are also visibly affected. This involves not only structural alteration but physiological effects as well, and even types of behavior. For example, the vitality of the organism is intimately related to some of the most trifling changes in superficial characters, and the productivity and fertility of the animal may be very greatly affected by mutant changes whose visible effects on the body are very slight. Conversely, it is probable that some of the genes that are very important in their influence on the physiological functions of the body produce side effects that show themselves in apparently trifling details of structure in the body whose constancy is due to the deeper effect of the gene rather than to some of its superficial by-products. It is quite possible that many of the constant features that distinguish species and varieties owe their constancy to their connection with deeper-lying effects than to their own survival value. This interpretation may help to solve a very old paradox, namely, that if natural selection has produced species it seems strange that the taxonomic differences between species have seldom a survival value.

The principal value of the mutant types, from the point of view of heredity, is that they furnish us with material from which we can arrive at conclusions as to the mecha-

nism of heredity. Furthermore, in studying the mode of inheritance of a mutant gene, we follow at the same time the history of the normal partner of that gene. It makes no difference, as far as the hereditary mechanism is concerned, whether the representative character for a given gene is a beneficial or a defective character. It serves our purpose as long as it survives under the favorable influences of confinement.

While most new mutant types are failures, so to speak, some of them approach very closely in surviving value to the wild types, and there may be a very few whose effects are beneficial. It is conceivable that a mutation might at times appear that was even an advance over the wild type, or at least, better adapted to a new situation—one that opened out new possibilities. When it is recalled that for millions of years those individuals that are best adapted to their environment have produced the next generation, it is to be expected that the wild type has become as nearly fitted to the world in which it lives as is possible for this particular organism. Or, to put the matter in another way, most of the possible variations have already been tried out and rejected as inefficient. The result is that most organisms are wonderfully adjusted to the world in which they live. The evidence from the recurrent mutations of *Drosophila* suggest that all the mutant types that turn up have been brought to the bar of selection over and over again and have failed. They recur because these are the most frequent changes that take place in the germ-material and they will continue to recur as long as the constitution of *Drosophila* remains the same as it is to-day, for the continued elimination of the recurrent types of defective characters that appear as mutants in no way guarantees that they will not reappear over and over again. They represent, so to speak, the bills that must be paid for the instability of the type of machine that makes each kind of organism as perfect as it is, but their recurrence is an extraordinarily rare event, and the chance that they contaminate widely the

germ material is in general very small. It is easy to exaggerate the effects of such results. The consequent drag may be so slight and be so quickly replaced owing to the enormous rate of multiplication of the rest of the individuals best fitted to survive, that the occurrence of defective mutant types is little more than a passing episode in the life of wild species subject to constant struggle with their environment. To what extent their occurrence may act as a drag when competition is removed, as it is to some extent in human society, will be considered later.

INHERITANCE OF THE CHARACTERS OF DOMESTICATED ANIMALS AND PLANTS

Plants and animals under domestication show innumerable characters that are inherited in the same ways as are those of *Drosophila*. The new types also behave in inheritance towards the original species in the same way as do the mutants of *Drosophila* toward the wild fly. It seems reasonable, therefore, to assume that these characters have also arisen by gene mutations. For example, the several recessive colors of sweet peas behave toward the wild type pea in the same way as do the mutant recessive types of *Drosophila* towards the wild type. The recessive colors of fancy rats and mice and guinea pigs behave as recessive to the wild gray animals. The pea and rose combs of fowls are dominant to the single comb of the wild jungle fowl. Some domestic types give intermediates when bred to wild types. There are also multiple allelomorphic genes in mice, rats, rabbits and in silkworms. There are modifying factors that affect the ear-length of the rabbit, the size of the corn cob, and the spotting of mice and of rats. There are sex-linked characters in fowls, and in fish, and in silkworms. Linkage, other than sex linkage, is also known for a number of domesticated forms, both plants and animals.

Thus animals and plants under domestication duplicate practically every kind of inheritance shown by the mutant

genes of *Drosophila*. No one familiar with this evidence is, I think, likely to doubt that we are dealing to a large extent, at least, with the same situation in both cases.

HUMAN INHERITANCE

When we turn to human variations we find characters that follow the same laws of inheritance; for example, blue eye color is a simple recessive to brown eye color. Albinism is recessive to pigmented skin. It is true we do not know in man what characters represent the "wild" or original type, but it is a fair presumption that primitive man did not have blue eyes, nor was he an albino.

The short-fingered or brachydactyl type of hand and foot is an excellent example of a dominant mutant type in man. Whether the different shades of brown hair color represent a series of allelomorphic genes, as has been suggested, but on quite inadequate evidence, or whether the different shades are mainly due to modifying factors, we do not know. Either interpretation may cover the facts. Similarly, for skin color, we have only guesses as to how many factor differences there are between the black skin of the negro and that of the white man. While it seems probable that these differences behave as do mutant characters in heredity, there is no proof at present as to how many factors are involved. Perhaps a negative statement may more nearly express our imperfect information on this question, namely, that there is nothing known at present to show that the inheritance of these pigments is not due to several Mendelian factors. Whether the negro has acquired a deeper color than his ancestors, and the white man has lost some of the color, and, if so, whether these gains and losses of color involve the same pairs of genes or quite different ones, we can not even surmise from what has been published.

There are three types of human inheritance that show clearly sex-linked inheritance, namely, color blindness, haemophilia and night blindness. Their inheritance is explained on the theory that a recessive factor is carried

by the X-chromosome, and there is cytological evidence that man belongs to the XX-XY type. It is true we know very little about the various types or degrees of color blindness (and of bleeders also), but for certain types at least the facts suffice.

That height in man is due in large part to hereditary elements is highly probable, but how many factors are concerned we can scarcely guess. Growth, as well as some other human characters, is affected by endocrine secretions of several glands. One of the direct functions of some of these glands is concerned in the postnatal growth of the individual, but this does not mean that the degree of functioning of these glands is not a heritable factor. On the contrary, it seems quite probable that the time of development and the rate of secretion may depend on such factors. If so, they are just as much genetic factors of growth as though they produced their effects in every cell of the body, rather than through the activity of a particular set of cells. In fact, there is nothing in the theory of heredity, as understood to-day, that precludes the possibility that the effects of some of the genes are produced on certain parts of the body through internal secretions made in other parts.

At present there are no cases of linkage known in man, despite the many pedigrees showing that certain characters are inherited. The large number of chromosomes in man decreases the chance that two or more genes lie in the same chromosome, but our failure to detect linkings may be also due to the very limited extent to which two or more characters have been studied in the same cross, for only in this way can linkage be detected.

From the evidence relating to the inheritance of characters in man, it appears that many mutant characters have here and there established themselves in the germinal material, and their reappearance from time to time is probably nearly always due to the mating of two individuals that carry recessive genes. The many human pedigrees of defective strains that we possess leave hardly any doubt on this point.

How frequently *new* mutants appear in man we do not know. If the human germ-material behaves in the same way as does that of *Drosophila* (and of other types) we may expect not only recurrence of known types by original mutation, but also the appearance of new types.

The survey of any list of known or partly known cases of human inheritance is likely to give the impression that variation by mutation leads only to the appearance of malformations, and pathological effects of the most varied kinds. I have pointed out that *Drosophila* mutant types present the same picture. It may be well, therefore, to pause here for a moment and look a little further into the situation.

The great majority of mutant types of *Drosophila* that have appeared could never establish themselves outside the laboratory under present conditions. If turned loose, they would be unable to survive, except for a short time. If one or another of them were crossed to a wild fly, its gene, if recessive, might to some extent infect the race and be carried along for several generations beneath the surface, so to speak. The character would reappear in its original form if two individuals carrying the gene met and mated, but the chance is very great that sooner or later the mutant gene would disappear from the germ material, because whenever it comes to expression it is discriminated against. It is only under the favorable conditions of confinement, and the absence even there of competition, that these mutants can perpetuate themselves.

THE ORIGIN OF VARIATION THROUGH VARIETAL AND RACIAL CROSSING

Within each species there often exist smaller groups of individuals known as varieties or races. These frequently occupy different regions. The differences that distinguish them are often trivial, but these may be, in part, only by-products of genes whose main effects are less obvious to the eye but important for the life of the species. So far as this holds, it may be said that each

variety or race has accumulated special characters of its own. Two such varieties may differ then in each possessing slightly different adaptive characters that show superficial correlated differences.

Since varieties can usually be crossed, and produce as a rule fertile offspring, it is possible by combining them to bring together in later generations every special feature that each may possess, but it is unsafe to assume, offhand, that, because each of two races possesses certain desirable traits, those traits brought together will give a double advantage. That they may be combined when they follow Mendel's laws is always probable; that when combined they will reinforce each other may be true, or may not be true; because the advantage of each may be due to other indirect or involved effects that make them advantageous when alone and not when combined. In such matters it is very unsafe to draw conclusions from insufficient experience.

There is another variation introduced by racial crosses that may be very important, namely, the question of fertility. It is generally believed by breeders of domesticated animals that inbreeding leads to sterility. That this happens at times appears to be the case from the evidence at hand, but that it is due to inbreeding *per se* is probably not true, because it has been shown for rats, mice and guinea pigs and flies that close inbreeding does not lead to a reduction in productivity, if, in each generation, the more productive lines are selected for propagation. On the other hand, when the breeder selects his stock for characters that have no relation to productivity, or worse still for characters that in themselves lower the productivity, there is a good chance that sterility may appear to a greater or less degree. If man is as mixed racially as the historical evidence seems to indicate, there is little danger that his productivity will be lowered by breeding within the races as they exist at present. The lowered birth-rate, shown by certain classes within the community, appears not to be the result of infertility but to environmental, prudential or economic factors.

There is another question that comes in here. Is it more advantageous to breed for variability or for uniformity? This question contains so many possibilities where man is concerned that a discussion of it would lead us far afield, but it involves a problem that is very important in any plans that propose to direct the future course of human evolution, and has been scarcely touched upon by those who discuss the question of human breeding.

As has been stated, some of the variations of domestic types are believed to owe their origin to the union of several wild varieties or races, for there are a few cases on record where such crosses have been made and great variability introduced into the later generations. Whenever such crosses do not involve so many differences that we can not trace them, we find a recombination of characters of the two crossed types. It follows, then, that while new combinations can be obtained in this way—by crossing wild varieties—there is nothing new in principle involved. Plants lend themselves more readily to varietal crossing than do animals, and a great many combinations have been made. In a number of plants, where the visible differences between the varieties have been carefully followed, they show Mendelian inheritance. In animals also there are similar records, in butterflies, moths, locusts, snails, fish, amphibia, birds, mammals. The combined evidence from all these sources indicates, if it does not prove, that the same laws of inheritance prevail that are found between mutant races. The conclusion may be expressed more accurately in a negative form as follows: There is nothing known in regard to the inheritance of characters that distinguish wild varieties from each other to indicate that they are inherited differently from the characters that arise by mutation.⁴

The human species presents a great number of groups that a zoologist would rank as varieties, although, owing

⁴ The question of interspecific sterility and that of the sterility of hybrids between species is intentionally omitted here, and the discussion limited to varietal crosses that produce fertile offspring.

to extensive migration of races within comparatively recent periods, the varieties are as a rule extremely mixed within each race. It is difficult to determine how far the variations that we observe are due to recombinations of characters that have arisen within each race and been brought together subsequently and how far the variations are due to mutations that have appeared within such mixed races. It seems highly probable that both processes have taken place. In a very general way it might be said that some of the defective types have probably arisen by recent processes of mutation, while some of the advantageous characters may have arisen through racial crosses. At the same time defective characters may equally well arise through racial crosses and advantageous characters by recent mutation. From a practical point of view these distinctions are, or may be, very important for any particular group of individuals, but from a theoretical point of view it is a matter of secondary interest whether the useful characters have arisen within the race where they are found or whether they have been introduced by crossing with other races already possessing them.

There is a further question relating to the study of inheritance of human characteristics that is not always clearly understood. We should expect, if all the various characteristics that we observe were inherited as simple Mendelian differences, that there would be evidence of it on all sides, even although we have seldom more than two or three generations under observation, and even although many of the combinations are not those that a geneticist would recommend. On the other hand, we find that it is the rare and extreme variations, often defective ones, that show, in man, most clearly Mendelian differences. Now the question that any one familiar with the situation will ask himself is, why is the inheritance of familiar, everyday characters not so obvious as is the inheritance of these rare anomalies? I think we can give, in large part, an answer to this question. The inheritance

of the anomalies is more apparent because they depend on single differences that have newly arisen and that are sharply defined, so that an individual that possesses them is distinctly marked off from all the other individuals of the race, while the commoner individual differences are generally complexes of characters—multiple factor cases, as we say—whose elements are unknown to us. Under these circumstances their study is very difficult, and will take much time and attention. One is tempted to think that many of these individual differences have arisen by racial crosses which have brought together large numbers of characters that have arisen independently, and while I think that this may very well be true in many cases, it is unsafe to refer all difficulties to this source, since within each race multiple factor differences are also known to exist. So, until these complexes can be sorted out into their Mendelian elements, they may well be left to the biometricians who have devised special methods for the study of such mass phenomena.

Of course there also enter into the situation the effects of the environment that no doubt play the same rôle in the development of human characteristics as in the characters of domesticated and wild animals and plants, but, as Galton long ago pointed out, the close similarity between identical twins furnishes evidence that nature (heredity) plays a far more important rôle than nurture (environment).

DO ALL HERITABLE VARIATIONS ARISE BY MUTATION OF GENES?

The evidence that we have so far considered postulates that the hereditary elements, the genes, arise as random alterations in the germinal material. It further appears that the great majority of these mutations do not give rise to characters that are beneficial to the organism. Nevertheless, it is assumed that at times a mutation may appear that happens to be of a kind that is beneficial in the sense that it better adjusts the animal or plant to its

old or to a new environment. Such a variation would be an adaptive one. From this point of view there is no relation between the *origin* of adaptive variations and the adjustment that follows. This account of evolution—which is the explanation offered by the theory of natural selection—seems to be unsatisfying to certain kinds of mind that seek for more intimate relation between the origin of new variations and the use to which they are to be put. Perhaps it is not going too far to say that these thinkers would like to believe that adaptive variations must from their beginning be, in some sense, purposeful, or at least that there must be some direct connection between the origin of an adaptation and its future use. Bergson's creative evolution is the best example of the consistent application of such a view to living things; for, Bergson begins with the outright assumption that living matter responds adaptively to new situations as they arise. The biological fact that many responses are injurious is ignored, or by a subtle argument is ascribed to a material obstruction to the creative spirit (*élan vital*). To the biologist the problem is one whose solution depends on the kind of critical evidence that can be found in its favor or opposed to it, and is not one to be settled *a priori* or by the introspective method.

A less mystical explanation of the origin of adaptation in animals is generally known today as Lamarckianism, although Lamarck only presented a systematized body of speculations relating to a widespread myth prevalent in the folk-lore of all peoples both past and present. According to this tradition any change in the body may reappear in the offspring. That this sort of transmission is possible was naïvely taken for granted. Darwin, in his hypothesis of pangenesis, made an attempt to give a materialistic interpretation as to the way the somatic change reaches the germ-cells. To-day, with more information concerning the relation of the germ-cells to the parent and also concerning Mendelian inheritance, the gap between the body and the germ-cells has widened

until at present the earlier speculations seem in flat contradiction to the most critical evidence that exists.

Nevertheless, since the time of Lamarck the theory of the inheritance of acquired characters has never been without a champion. Even to-day the transmission of acquired characters is widely believed in by the populace at large and by those who are not informed as to the present nature of the evidence on which such views rest. It is also looked upon sympathetically by a small number of scientific men whose specialties lie in historical fields of research. There are also a few other scientific men who are devoting themselves to the study of the possibilities of the transmission of somatic and environmental effects on the germ-cells. These men fall into two groups, first, those who have chosen their materials of such a kind that they do not lend themselves to quantitative methods, hence there enters into their conclusions a large personal equation; and, second, those who have fulfilled, as far as possible, the requirements called for in the study of such problems. It is, therefore, not without interest to note that while the former workers vehemently advocate the theory of the inheritance of acquired characters, the latter have brought forward evidence that opens up the possibility of a very different interpretation. It is to the results of the latter that we may now turn.⁵

Guyer found that if the lens of the eye of rabbits is ground up and injected into fowls, and if later the blood of the fowls is injected into a pregnant rabbit the eyes of the young rabbits when they are born may be defective. He suggested that the rabbit's lens has produced an antibody in the blood of the fowl and this in turn affects the developing young of the rabbit that received the injection of fowl's blood. He records that some of the later offspring from the first young with defective eyes also showed eye defects. Now if these results are not merely coincidences, the most plausible explanation is that the

⁵ No attempt is made here to review all the evidence. The cases given are chosen only for the purpose of illustration.

germ cells of the uterine rabbits were also affected or injured by the injected fowl's blood. Several considerations seem to support such an interpretation. First, the specific nature of the result is illusory, for a closer reading of the evidence shows that the eyes of the later generations are affected in all sorts of ways. In other words the experiment was ostensibly carried out because of the specific nature of reaction of lens substance, but the result shows that all kinds of eye defects were produced or at least appeared.⁶ Second, it is known that many environmental changes that affect injuriously the course of normal development of vertebrate embryos are apt to produce eye defects. These are not supposed to be specific effects, but rather that the eye development is more easily injured than almost any part of the embryo; for eye defects have been produced in guinea pigs by the injection of naphthaline, and these defects seem to be similar in a general way to those described by Guyer in his rabbits and by Stockard in his alcoholic rats. Here there can be no question of a specific reaction.

Stockard carried out a prolonged series of experiments on the effects of alcohol on guinea pigs. The guinea pigs were treated by placing them in closed tanks over strong alcohol. They breathed the air saturated with alcohol, and after a few hours became completely stupefied. The treatment was carried over several years. Some of the guinea pigs were bred while undergoing treatment, others only at the end of the treatment. The results were essentially the same. Many young were aborted or absorbed, others were born dead, others showed abnormalities, especially in the nervous system and eyes. Only those that themselves showed no defects could be bred. From these, abnormal young continued to appear along with other individuals normal in appearance. In later generations abnormalities continued to appear but only from certain individuals.

⁶ It has not been shown that these were secondary effects resulting from a defect in the lens.

If we examine the pedigrees of the alcoholic series there is no evidence that the results conform to any of the known Mendelian ratios. Moreover, the varied localization of the effects shown by the abnormals is not of a kind that resembles what we meet with when single gene-changes are involved. On the other hand they have many points of resemblance with the kind of changes that we are familiar with in experimental embryology when abnormal development is brought about by treating eggs with toxic agents. Stockard has called attention to these relations, and interprets his result to mean that an injury of some sort to the germ-cells has been produced by the alcohol—an injury to some part of the machinery that is involved in heredity. The effects are localized only in so far as that they pertain to those parts of the body that are most sensitive to any departure from the normal course of development. These parts are most frequently the nervous system and the sense organs.

More recently Bagg has carried out a series of experiments on the effects of radium on pregnant mice and rats. When the dosage is properly administered, the young mice in utero may develop abnormally. When examined before birth many of them show hemorrhagic areas in the brain and cord, or elsewhere (especially in the leg rudiments). Some of these embryos die before parturition, and are absorbed, others are aborted. Still others are born alive and some of these survive and many procreate. The offspring often show serious defects in the brain or in the appendages. One or both eyes may be defective. Both eyes may be absent, or one only may be absent, much reduced in size. Bagg has bred some of these mice and finds that they produce many abnormal offspring that show defects similar, in a general way, to those induced directly in the original embryos.

How shall we interpret these experiments? Has the radium first produced its effects on the brain of the developing embryo causing defects, and is it owing to the presence of these defects that the germ-cells of the same

embryo become affected? There is an apparent objection to this interpretation. We should expect when the brain alone is affected, the next generation should show brain defects; when the eye is the principal organ affected, the next generation should show only eye defects. So far as reported the results are not like this, for a mouse with abnormal brain and full-sized eye may produce offspring that have defective eyes. In other words, there is not here a specific effect, but a general one.

The other interpretation is that the germ-cells of the young mouse in utero are affected by the radium. When, in turn, these germ-cells produce a new generation the individuals are defective because the same organs whose normal development was most disturbed are the organs that are most easily affected by any alteration in the course of development. They are, in a word, the weakest or most delicately balanced phases of development, and therefore the first ones to show the effect of any departure from the normal course of events. This is, I think, at present the most plausible explanation.

It has been intimated that those reagents or conditions that bring about a modification in an organ of the body may also affect the gene or genes in the germ cells that are responsible for the development of this organ. A new term begins to be heard to express this imagined relation, namely, somatic induction. I can not now stop to discuss the relation here implied, but I can at least point out that such a view often rests on an entirely erroneous conception of the relation of character and gene, for the character is not the product of any one gene, but of all the genes that affect that part. This interpretation is far removed from the one implied in somatic induction that savors of the old ideas of Weismann concerning determinants and characters. Even if it turns out to be true that the change in one gene, that leads to a definite change in some peculiarity of an organ that has developed under the influence of that kind of gene, is a specific chemical change, there are at present no grounds

for the assumption that the chemistry in the two cases is the same. On the contrary in the light of the present evidence it would be most extraordinary if such were the case.

SOCIAL AND ECONOMIC INHERITANCE IN CONTRAST TO BIOLOGICAL INHERITANCE

While there appears to be no sufficient evidence that justifies us in accepting the theory of the inheritance of acquired characters, *sensu strictu*, either for animals or for man, there is another factor in human progress that plays an enormously important rôle in man's life and progress. I mean his social and economic inheritance. Here the acquired experience, as well as the products of the experience (property, machinery, customs, myths) are transmitted from one generation to the next and are inherited by the latter. In this respect man has reversed the old order of nature. He may not inherit the bodily or the mental characters that his parents have acquired through training, but in another way he inherits the results of their experience. Owing to his possession of a wonderful memory, of his acquisition of speech, and then of the art of writing and printing, and last but not least his power of learning which his prolonged childhood enables him to endure, accompanied by an overlapping of generations, he is able to endow each successive generation with the acquirements of the preceding ones. Man's rapid social evolution, cutting ruthlessly across the slow course of his physical evolution, may at times lead to conflicts between the two processes. So great and rapid has been the effect of his economic and social success, it has appeared to a few observers that his physical evolution has come to a standstill. I do not share this opinion. It is an interesting question, nevertheless, to what extent the social evolution of man has affected his biological evolution.

If we take the long view we will see, I think, that while man's social evolution has come here and there into con-

flict with this physical and mental evolution, yet the importance of the latter is not set aside, but continues to pursue its slow but sure course. A few imaginary situations will serve to give some idea of the contrasts I have in mind. If as a result of religious conviction most men went into monasteries and most women into nunneries, the progress of the race might be set back for some time, provided these were the individuals best suited to bring about the progress of the race in a biological sense. It might be argued, of course, that the very fact that these individuals reacted in this way is in itself evidence that they were not suited to continue the race; but the argument is dangerous since the same individuals might equally well, under a different influence, be those to respond to another more generous conviction that greatly benefited the progress of the race. However this may be, there is in the former possibility an obvious conflict between social and biological evolution.

To take a less extreme case no one will dispute, I suppose, the statement that if in any country a particular caste or class does not reproduce its numbers in each generation, it will disappear by a process of natural selection. But if thereby the intellectual or creative productivity of such individuals were increased the results might be beneficial to all the rest of the race. Those nations that profited by this specialization, that inherited, so to speak, some of the benefits accruing from the activity of this postulated semi-sterile class, might be expected to advance more rapidly, other things being equal. The loss of a few breeders might be more than compensated for by the gains resulting from their release from the burdens of propagation. How far inter-racial competition is or may be affected by specialization of this sort no one, I suppose, will pretend to state. Let it not be inferred, however, that those social classes that do not reproduce their quota of offspring do so always from altruistic motives, or that they make increased returns in other directions, but this affects the results only indirectly. The

point at issue is whether on the average there is a compensating gain for the failure to breed. This is a problem for the economist.

As the population increases and as means of transportation develop, the opportunity for the spread of contagious diseases is greatly increased. The resistant individual survives, the non-resistant dies. This is natural selection in the biological sense. But suppose with modern sanitation we remove the cause of the disease, as has been done for small pox, for malaria and for yellow fever. Has natural selection come to an end in this respect? Obviously yes, since for those particular diseases there would no longer be any need for biological adjustment; the disease having been removed. Henceforth susceptible and non-susceptible persons are on the same footing. It is a distinct gain to the race to acquire its immunity by elimination of the disease, rather than by the elimination of human beings. Biological selection here has ceased to have a selective value for this particular disease because the disease has gone, but those races that are advanced sufficiently to discover and put into force the regulations that eliminate the contagion, are benefited, in comparison with those races that do not act in this way. This is not the abrogation of natural selection, but rather the process raised to a higher sphere.

I have attempted in a general way to go over some of the aspects of human inheritance in the light of recent work in genetics. In the case of man, I have pointed out that we use the word inheritance in a double sense. In a biological sense it means one thing, in a social sense it means something quite different. While these two aspects of human heredity have seldom been confused by those writing on the subject, nevertheless, I can not but think that our familiarity with the process of social inheritance is responsible, in part, for a widespread inclination to accept uncritically every claim that is advanced as furnishing evidence that bodily and mental changes are also transmitted.

I have also pointed out how social progress may at times make conditions more favorable for biological evolution, and at other times retard it. To study the interaction of these two influences on human progress, to bring them into accord so far as possible, is one of the most interesting and difficult problems ahead of us. The geneticist alone can not hope to solve such a complex problem. The psychologist and the physiologist and the pathologist are needed, especially in the diagnosis of those characters that belong properly in their special fields. The failure of critical diagnosis accounts in large part for the disrepute into which some of the work on human mental traits has fallen. Students of human behavior and anthropologists also will no doubt have something to contribute. Economists and statisticians will be needed; for at present, there are, in man, many inherited characters that furnish only raw data. I do not suggest that these experts form a committee to formulate a program. I only mean that competent specialists are needed in each of these fields to push forward scientific investigation—since other methods have signally failed—and that they keep an eye on what is being done in other fields. The outlook at present is encouraging. Men have awakened to the fact that a scientific study of human progress is possible. I believe that they will not much longer leave their problems in the hands of amateurs and alarmists, whose stock in trade is to gain notoriety by an appeal to human fears and prejudices—an appeal to the worst and not to the best sides of our nature.

ANY HEREDITARY CHARACTER AND THE KINDS OF THINGS WE NEED TO KNOW ABOUT IT

DR. OSCAR RIDDLE

CARNEGIE STATION FOR EXPERIMENTAL EVOLUTION

No one seems ever to have written the results of a serious inquiry as to which are the distinctly different kinds of knowledge that will be required for the adequate comprehension of a (any) hereditary character. It is possible that studies in heredity have lost and now lose something of perspective and of balance by the absence of some sort of gauge against which actual accomplishment in this subject can be measured against the total necessary accomplishment. The older and more inclusive science of biology has made far more definite and helpful classifications of its constituent aspects—as applied to organisms and to groups of organisms—than has heredity. These divisions or aspects of biological science—comparative anatomy, systematics, biochemistry, paleontology, behavior, embryology, evolution, pathology, ecology, micro-anatomy, physiology and distribution—are at once frank recognitions of the kinds of knowledge necessary to a comprehension of the organism, and of the limited scope and value of any single type of information. Heredity, or evolution, like biology as a whole, possesses an integrity which upon examination immediately dissolves into diversity. It is a crystal of many facies. The first purpose here is to attempt the identification of the radically diverse aspects presented by any single hereditary character. This attempt is the more opportune because some recent developments in sex studies now make it fairly clear that one or two new or hitherto imperfectly conceived aspects of a hereditary character can be identified as distinct and utilizable aspects of any hereditary character.

In addition to matters of theory, investigations current in heredity are confronted by a condition. The onrush of data and facts now proceeding from the world's laboratories of genetics would seem to bring assurance that the province of heredity and evolution will soon be covered with a body of precise and definitive knowledge—quite sufficient perhaps to satisfy the accumulated curiosity of two generations for broad and positive knowledge in this field. May a worker within this group, which conceives its work to be the study of “heredity and evolution,” without seeming too ungracious, raise a question as to whether our wealth of published contributions—and the investigations we are pursuing, directing or encouraging—really covers the required and now accessible range of information on heredity and evolution? Are we, as individual builders of a house of science, assured that our work is upon all its walls and foundations? Is it possible that it is rather a slender tower than a symmetrical home that engages our very effective efforts? Is this possibility at all worth considering? Does the sweeping cloud of data, great in volume but limited in kind, at all affect our vision of other important but missing kinds of facts? Do our enormous local successes mean a general advance all along the line? In connection with a principal purpose stated above it has seemed obligatory seriously to examine, however briefly, the point involved in these questions.

No one will momentarily doubt the great value or the gratifying volume of knowledge now being obtained in that part of the field of heredity known as genetics (breeding and cytology). This is all entirely obvious; and, in a statement condensed to the point of running other risks of misunderstanding, the writer trusts he may omit any review of actual accomplishment in this field without subjecting himself to the charge of either overlooking or of being unaware of its invaluable contributions. In general, this discussion is to emphasize limitations rather than accomplishment. Examination of our first point involves an estimate as to how far the data of

genetics include a real or a complete knowledge of heredity and evolution. Besides dealing with the limitations inherent in the types of information now rapidly accumulating, it will be necessary to characterize and to consider specifically each of the additional kinds of information necessary to an adequate understanding of any hereditary character.

The conception presented here also involves the proposition that our knowledge of heredity and evolution will become essentially complete when we shall have learned all the necessary kinds of facts about one, *any* one, hereditary character; but that an infinite number of facts of the few (practically only two) kinds now being actively and most successfully gathered, can never give us more than an unfinished fragment of the knowledge necessary to a comprehension either of evolution or of any hereditary character.

The following diagram lists these wholly distinct, and now identifiable, aspects of any hereditary character:

Any Hereditary Character					
a	b	c	d	e	f
Origin	Complete ontogeny	Foundation and localization in gametes and zygotes	Mechanism of distribution in gametes and zygotes	Intimate nature	Control or transformability in ontogeny (and phylogeny)

It may be well to state clearly and at once that some facts drawn from many or all branches of biological science, as well as from other sciences, will have to be utilized in obtaining the requisite knowledge concerning any one of these six aspects of any one hereditary character. For example, physics, chemistry and most or all of the groups of biological science mentioned in an initial paragraph may be involved in what is required for a complete knowledge of the *origin* or the *complete ontogeny* of a character. But the entire body of knowledge of any one of those science groups would probably not be involved in supplying the required information on the origin of *all* hereditary characteristics, and this requirement would

be enormously reduced if applied to the case of any one such character. Certain parts of various branches of science may thus later become a part of the subject of heredity in precisely the same way as a part of cytology has already become a part of genetics.

It should also be made clear that we neither mean to state nor to imply that only *c* and *d* of the above classification are now receiving any attention by geneticists. The point raised here is that these aspects only are receiving anything approaching the share of active work which other aspects of heredity should now receive. Most geneticists were biologists before they became geneticists; many workers have realized the need of one or more additional kinds of information concerning the characters with which they work, and to one or another extent they have sought to supply some parts of this information for their own material. In large measure it is in consequence of those efforts that it now seems desirable and practicable to inquire specifically into which are the distinct kinds of knowledge really required for the comprehension of a character. This gathering of scattered and unrelated bits of such information among many characters has rendered a further service in that it now enables us more clearly to see the great importance of having all the necessary information gathered for some one—any one—character.

ORIGIN

Our classification or diagram gives first place to the *origin* of hereditary characters and factors. Under origin is of course ultimately implied: *Demonstration of occurrence*, with data capable of distinguishing the pathological or abnormal from that of constructive and evolutionary value. Changes of pathologic or abnormal origin can certainly be inherited, but they have a quite doubtful status in creative evolution. Such changes, though hereditary, may very little resemble the actual “origin” of characters. *Cause*, with the few or several sequences in-

volved. *Method*, with the types of functional and structural changes concerned in the rearrangements of matter and energy. *Place*, with reference to other hereditary units and to the organism. It does not yet seem entirely beyond question that all characters ultimately of evolutionary value arise *first* in germinal tissue. *Time*, specific for individual case (or, much less important, historic appearance). It is clear that the attainment of complete knowledge here will be a difficult matter.

All will grant the great theoretic importance and need of this kind of information concerning any specific character. It seems to be true, however, that our definite knowledge dealing with what is certainly the origin of characters of evolutionary value is still essentially limited to evidence for the relative time and order of the appearance of a number of these in evolutionary history—a minor contribution to this problem by paleontology and comparative anatomy. In addition, it is possible, but by no means certain, that we already have from current investigation in genetics some—though only a part—of the highly important facts that should sometime be found for the origin of characters as they arise in evolution. Some investigators in genetics have urged that certain types of mutation, and still other cases involving observed chromosomal rearrangement, have a right to be considered as actual origins of such new characters. But many or most biologists, including some geneticists, are quite unwilling to concede that proposition. There are weighty objections to considering either hereditary losses or gains following losses within a species as origins of things really involved in evolution. For present purposes it can be said that even if these cases be granted the status of such origins the amount of knowledge they bring concerning their origin, though of very great importance, is notably incomplete. We may perhaps well doubt that any line of investigation now in use will give us the information we most seek concerning the origin of any specific character. It is quite possible that the required information—if attainable at all—awaits the development

of essentially new avenues of approaching the problem of heredity; or probably of these combined with present methods of genetic study. The very special theoretic and practical importance of this deficiency in our knowledge may well give us composure while viewing contemporary triumphs in other related aspects of the study of heredity.

COMPLETE ONTOGENY

A second type of information (*b*) we have characterized as the *complete ontogeny* of a character. The requisite data here involve knowledge of each step of the action of the hereditary factor toward the differentiation of the corresponding hereditary character. Concerning this immensity, stretching from and preceding the quiescent gamete through embryonic stages to the finished—if ever finished—adult character, we now have only scattered traces of information. More regrettable still, nowhere may one find a considered program for bringing into existence this type of information for any hereditary character. Many items of such information have indeed been obtained, in a few cases as a real part of a genetic study, but largely as by-products of studies in embryology, biochemistry and chemical pathology. Most of even these few items can not be said actually to have been incorporated into the science of heredity.

It is perhaps well to cite a specific case in order to make clear our meaning and to attest the present practicability of such studies. In the complete ontogeny of a character its form-expression in the embryonic series is perhaps incidental or quite negligible in the case of most hereditary characters; in a few of them it is probably of real importance. The most necessary data in work of this type will usually involve chemical aspects of ontogeny. In illustration one may cite a promising bit of information obtained in studies on the liver of the human foetus. Some of the steps by which one of the adult functions of the liver is attained have been disclosed through the observation that the purin bodies (protein constituents)

are there broken down in successive stages by enzymes which first appear in an orderly time sequence. This order of enzyme production being—guanase, adenase, xantho-oxidase. That these represent orderly and successive steps in the ontogenetic development of a definitive function of the adult liver is made clear in these studies. Also, in this particular case, the far-reaching hereditary significance of this sequence is made further evident by the fact that the order of ontogenetic sequence of these enzymes is probably the order found in animal phylogenesis.

If another illustration were required it could be shown that the differentiation of the sex character also offers a quite favorable opportunity for studies of this nature. We shall here note only that "metabolic rate" is a thing susceptible of measurement in all stages in one or another organism; that the various form-expressions of sex in the embryonic series are perhaps as well known as for any character; and that in vertebrates much work has already been done on the nature and developmental effects of the substances elaborated by or in association with the gonads. These facts, gathered hitherto by workers in different fields and with most varied aims, supply a good beginning for a study of the complete ontogeny of this character.

There can be no doubt that this fairly obvious and almost entirely neglected field—now scarcely recognized as within the province of research in heredity—is a wholly essential part of the required knowledge of any hereditary character. It would, therefore, seem to be important that present workers in heredity recognize the need of adequately and permanently establishing this type of work as an essential aid to the problem of development—with heredity as the point of interest and as the aim of such study. At present we must rely upon chance information—the by-products of studies in pathology, embryology, biochemistry or medicine. Further, along with this recognition it would seem necessary to begin the training of investigators who, with new methods of study

and independent outlook but well reinforced by interest and training in heredity, must in large measure be responsible for developing our knowledge of this aspect of heredity. In the task of securing and training these recruits it seems clear that geneticists, botanists and zoologists will first need to look to the breadth of training given within these subjects; and that they shall then have to secure the aid of their colleagues in chemical aspects of biology. A wide and thoroughgoing cooperative effort is here clearly required in order that we may hope later to attain this necessary information for any hereditary character. The circumstance that numerous fragments of data are known for different parts of the ontogeny of various characters does not even partially supply this need.

LOCALIZATION AND DISTRIBUTION

The *germinal foundations* of hereditary characters (*c*) have been so successfully examined during two decades of revolutionizing study and form so conspicuous a part of the contribution of modern genetics that, as already noted, this subject does not call for consideration here. This is one of the two from a total of six essential aspects of heredity that can be said to have received its share of deserved attention.

The *mechanism of distribution* of hereditary characters (*d*) has also been so successfully studied, and the results employed with such brilliant results—its testimony having been brought into striking consonance with that presented by the immediately preceding type of study (foundation in gametes)—that this body of knowledge also stands in no need of emphasis. The mechanism of distribution of hereditary characters is then the second and last of the six essential aspects of heredity that can be said to have received its proper share of attention.

These two last-named aspects of the subject may indeed be considered the mountain of fast accumulating information in the wide otherwise uncultivated province of heredity. To a certain extent our additions to this

mountain proceed, at least in many quarters, as though we conceive nearly all else within the horizon as a fallow and negligible area. It now seems in the interest of further progress to question this view, or at least this our actual method of procedure. On the other hand, the inference should not be drawn that all the major facts concerning the foundation of characters and the whole story of the mechanism of their distribution have already been obtained; nor should there be any slackening of effort on the more inviting unsolved problems in these two aspects of the subject. Besides all this there apparently remain many tasks, even within this restricted field (*c* and *d*), for the accomplishment of which present methods of study may prove inadequate. In a large part of what has hitherto been learned (or has taken the form of classifiable knowledge) the chromosomes are principally involved. But in the case of several of the most important features of individual development the "germinal foundation" is still conjectural or quite unknown; and there are features of the organism which are not, and others which probably are not, based upon any familiar form of "distribution or segregation." Only future investigation can disclose the facts for these particular cases.

The cases referred to above are made specific in the following examples: The germinal basis of polarity and bilaterality; the differentiation of the main body regions (head, thorax, body); the difficult fact that the effects of a gene are largely confined to localized areas, despite the circumstance that all the cells of the body have a common chromosomal equipment; and finally, the fairly obvious circumstance that the most essential of all the properties of the organism—the fundamental properties of living matter—can not be conceived as at all subject to segregation. In this latter case we must conclude that segregation is impossible, or else we must profoundly modify our definitions and conceptions of living matter itself; for, if these properties do segregate it follows either that particles of living matter exist with one or another such thing as irritability, respiration and assimilation left out,

or that such segregates involve only non-living matter. To urge, for example, that for "protoplasmic respiration" there are too many genes and these too widely represented in all the chromosomes to permit our ever seeing any evidence of segregation; or indeed to assume that any gene whatever exists without itself actively exercising this property, seems to resolve an established principle into an absurdity. Neither segregation, cross-overs, non-disjunction nor duplication can apply to the fundamental properties of living matter.

INTIMATE NATURE

Two other and additional aspects of heredity remain essentially undeveloped (*e* and *f* of diagram). It is not easy to make clear at once exactly what is meant by the "intimate nature" (*e*) of a hereditary character (and of its factor basis)—so unfamiliar is this conception to the language of heredity and evolution. But is there not a trace of humiliation in the circumstance that the state of advance in our science is such that any novelty or unfamiliarity attaches to a term like the "intimate nature" or the "properties" of a hereditary character (or factor)? Is there something other than matter or energy, and their various forms and transformations, involved in any such factor or character? Elsewhere in science workers with the forms of matter or the forms of energy, after assurance of their presence or existence, seek *first* to get at the identifiable properties of the bit of matter under consideration. In other fields of investigation iron, sugar, mercury, alanin and adrenin are subjected first of all to disclosure of their properties—their intimate nature. The investigator of heredity now reports variety or differences in his substrates; their localization; and he occasionally refers to the quantities or proportions of his substrates—his sugar and his mercury. But any two of his sugars may be equally regarded as iron and alanin; the effects of any gene being estimated solely on the basis of the total accomplishment of all other genes when that particular gene is present as contrasted with what

they do in its absence (or in its alternative representation). As to other intimate differential qualities of the genes we hazard no inquiry. Admitting this procedure, what further might we do about it? A plain suggestion seems to be: First, to recognize this neglect as a real and evident weakness of the present restricted attack on the problem of heredity; a weakness which merits the attention of individual investigators and of laboratories. Second, to prepare ourselves or some of those trained by us—again necessarily enlisting the cooperation of our colleagues in the requisite sciences—to enter and develop this essential part of the study of heredity.

But are we in a position reasonably to hope for success in this endeavor? To this question most geneticists will now doubtless reply in the negative; and certainly only that reply is possible for those who have not carefully followed other work in heredity than that most prevalent in genetics (*c* and *d*). On this point the writer must state his own conviction that the accomplishment of this precise thing is now becoming clear in the case of one hereditary character, namely, sex. Investigations of the past few years have supplied a large and varied body of evidence that the sex differential, as between male and female, is based upon initial differences of metabolic rate in the gamete or germ stage; that whether ova or sperm are formed within the developing organism depends primarily and continuously upon whether a higher or lower rate of metabolism is maintained in the developing organism from the gamete stage onward through the period of its own production of germ cells; that this intimate metabolic state *can* so definitely dominate the sex factor and the sex character as to determine in fine detail the expression of this most widely expressed character, irrespective of the type of factorial foundation in germ and zygote. The completeness with which this metabolic rate both *replaces* and *supplements* the impulse of the factor itself; and the circumstance that in metabolic rate we are not dealing with an external agent, or with a stimulus whose seat of action is unknown, but with the seat and

actuality of action itself—all this provides evidence that this particular factor impulse and metabolic rate are the same kind of thing. In this case, therefore, we now probably have some knowledge of the “intimate nature” of one hereditary character. It is a corresponding or similar type of knowledge that is needed in the case of any hereditary character.

In this connection it may be noted further that, if the above conclusion is correct as to the “intimate nature” of the sex character and the sex factor, we are in this case also in a very favorable position to attain an unusual view concerning one phase of the *origin* of this particular character. It has been pointed out that sex in the living world has originated independently hundreds, perhaps thousands, of times. How does it happen that these numerous independent origins of sex all give us essentially similar pictures of the two sexes? Would it not greatly assist us to an understanding of this matter if we could know the thing out of which sex differentiation arises? And if at the same time we might know also that this same thing is of wide distribution—as widely distributed in fact as are organisms themselves? It was noted above that these useful facts are becoming available. If all sex rests primarily and fundamentally upon metabolic level or rate, then all the numerous cases of independent origin of sex arose in organisms which necessarily already had one or another rate of metabolism prior to the differentiation of the two sexes from a unisexual condition. All these organisms possessed exactly one and the same thing, or kind of thing, out of which to form or differentiate the sexes. It seems a safe conclusion that it is now possible to undertake a study of the “intimate nature” of some characters and of their factors beyond the effects on development observed in their presence or absence as noted above, and that this fifth aspect of the study of a character is essential—like the preceding aspects of the problem—to a real understanding of any hereditary character.

Since we emphasize the special importance of obtaining adequate knowledge of all the six aspects of heredity on some one character it becomes desirable to inquire whether there is any one single character particularly favorable for all these types of study. In the writer's opinion sex is one of the most favorable characters for this purpose. Something of the status of the sex character with reference to four of the six kinds of knowledge is elsewhere mentioned. Concerning its position in the remaining two types (*c* and *d*) it is sufficient to recall that for no other character so well and so widely as for sex has it been possible to identify a visible "germinal foundation"; and that the "mechanism of distribution" of the factors underlying and normally guiding the development of sex characters is as well understood as that for any known character. It may further be said that experience indicates that in no one animal or plant species may we hope to study advantageously all the aspects of sex or of any other character. Once a particular character is selected for study each of the six kinds of required knowledge should be sought in whatever organism lends itself best to the specific aspect taken for study. It is practically inconceivable that these six radically diverse kinds of problems are all most favorably presented in any one type of organism.

TRANSFORMABILITY

The final and the practical aim in the study of any and all aspects of any hereditary character may be said to be for its control or transformability—in ontogeny at least, in phylogeny if possible. Is this aspect of the problem now amenable to study? Again we note that recent developments in the study of sex fully demonstrate that this particular hereditary character can be completely transformed to its alternative state, even in adult higher animals. It follows that, since the knowledge acquired on one truly hereditary character (sex) now enables us in the case of some higher animals to force this character to develop into its alternative or opposite form, the experimental con-

trol over all hereditary characteristics of this type becomes theoretically realizable and possible. No such character—physical or mental, in man or other organisms—can now be considered irreversible. This can only mean that the full development of a complete science of heredity will have included the control or transformation of characters in ontogeny as a definite part of its aims and attainments.

The application of this control and transformation not merely to ontogeny (the character) but to phylogeny (the factor) in addition might of course put us in a position to control or direct evolution itself—the ultimate goal of our science. Whether in fact this most important power resulted would depend upon the kind of heritable change effected; that is, whether what we succeed in introducing into the race is something abnormal and incapable of further progress, or is a typical and normal fundament as capable as were its precursors of further progressive creative evolution. In the one case we should be taking a magnificent part in creation; in the other, perhaps only facilitating factorial disorder or disease. In the event of a real success here this aspect (*f*) of heredity would meet and be resolved into one phase of the first-described aspect—the origin (*a*)—of a character. A few attempts to induce one or another heritable change by some specific treatment have been and are now being made, and all recognize that much additional work of this character is needed. The apparently valid heritable changes hitherto found do not seem clearly to involve a particular factor or group of factors, they are probably all of the nature of abnormalities, and they thus far introduce no desirable or promising thing into the heredity of the organism; yet, those very few cases in which the abnormalities can be assuredly associated with a specific procedure or treatment seem to supply a new and most valuable kind of fact. This aspect of heredity, though in all respects quite unsuccessfully studied until very recent years, already promises to receive a share of attention in the immediate future. The same can not be said, how-

ever, for the control of heredity in the ontogeny of man and animals.

Many will be inclined to consider lightly this power to control alternative characters in ontogeny. It may, nevertheless, be well for students of heredity to reconsider this matter in the light of a demonstration cited above. As an aid to such reconsideration it may be suggested that the group of sciences we now call medicine is built upon and takes its value from a measure of control perhaps less far-reaching and less advantageous to the human race than that involved in the control of alternative hereditary characteristics during the development and life of the individual. Medicine also chiefly deals with individual (ontogenetic) life. It sometimes preserves or rehabilitates life during many years; but more often its service—aside from sanitation and hygiene—is limited to aiding our resistance to pain or disease during only hours or days of our life. The complete control of (ontogenetic) heredity, however, would give to all men during all the days of their lives the greater resistance to disease, the predisposition to the longer life, the more advantageous stature, the higher level of intellect, the more desirable of all mental and physical states having allomorphic representation within them. There is perhaps little that is imminent here; but the range of development of a complete knowledge of this aspect of heredity is the point discussed. It would seem unfortunate if our science should long overlook the great human value and the educative and other responses of mankind to any practical advance in this field.

In conclusion, as workers in a common science—if in fact we are all really aiming at an understanding of heredity and evolution—may we not profitably consider the question which every laboratory, great or small, must raise in determining its policy? Are we as individuals, laboratories and institutions to make our main effort the filling in of details concerning conceptions or principles already fairly established, together with additional conceptions of a similar kind which will doubtless follow?

Or shall we consider it equally important, at least of much importance, to wedge our way into essentially new but now recognizable aspects of the general problem which must disclose kinds of fact new and different? It would seem that an adequate development of our knowledge of heredity and evolution requires that at least a few individuals and a few laboratories should now take the latter view and accept the duty and privilege of the development of one or another of the following neglected aspects of the study of a hereditary character: Its complete ontogeny; its intimate nature; its control or transformability.

SUMMARY

Present studies on heredity and evolution offer what is mainly a two-sided attack on a many-sided problem. An attempt to identify the radically diverse aspects necessary to the comprehension of any hereditary character, together with a concrete examination of these neglected attackable aspects of the subject, brings into clearer view the inadequacy of the present attack. Some of these deficiencies are such as can be adequately met only through a wide interdepartmental cooperative effort. On contemporary students of heredity and evolution and on laboratories devoted to studies in this field rests the responsibility of obtaining this cooperation, and of so directing some of their main efforts that the results of this cooperative effort may soon be attained. Where individuals or laboratories are already prepared to conduct this type of work it should receive immediate, active and encouraging support. Our knowledge of heredity will be more advanced by securing all the kinds of fact necessary to an understanding of some one—any one—character than by a duplication of much information of a few kinds on many characters. At the present time sex is one favorable character for such a comprehensive study. Heredity, as a branch of science, is assuming new aspects which give it an ever-increasing human value and a greatly increased human interest.

A HYPOTHESIS OF "VALENCE" IN HEREDITY AND EVOLUTION

F. M. GETZENDANER

EVEN the smallest detail of heredity depends upon the behavior of those infinitesimal chromosomal units, the genes. And these genes always appear along the nuclear threads in linear series, each of its own specific kind and self-perpetuating by growth and division. They are "of definite number, separated by fairly constant intervals, and are arranged in a definite and invariable serial order."

There is something about the above declarations of Professor Edmund B. Wilson regarding the structure of the cell that reminds one of Sir Ernest Rutherford's description and Bohr's graphs of the atoms. Rutherford says: "The atom is naturally the most fundamental structure presented to us. Its properties must explain the properties of all more complicated structures, including matter in bulk." May we not also say, "The cell is naturally the most fundamental organic structure presented to us; its properties must explain the properties of all more complicated organic structures, including life in bulk, heredity and evolution."

There is something about the *stability* of species, all separated by appreciable gaps, that reminds one of the stability of those atomic organizations which constitute the elements, all separated by gap intervals which can be expressed in terms of number and weight.

Can we not see, even though vaguely, some of the elements of the laws that are made imperative by conditions in the organic world? A simple trial-and-error mode of species origin through environmental influences, or the same mode through chance combinations of germinal factors, seems untenable in its final consideration because of the remoteness of the possibility that chance in any

length of time, no matter how great, could ever bring about a combination necessary to produce even the more simple of the plant and animal species. Either method of species origin, if operating alone, must operate under the law of probabilities.

It has been said that we may as well expect an explosion in a print shop to produce Hamlet as a combination of chances befalling the chemical elements to produce a lily or a lobster. Or, we may add, the chance mingling of negative and positive charges of electricity to produce lead, or the chance mingling of the atoms to produce a chemical compound. All these alike are inexplicable without the property of mutual attraction among some of the factors and the absence of it among the others, and a state of stability due to a law of definite proportions and definite structural arrangement.

An illustration that has been used in explaining the formation of chemical compounds must be applicable in the organic world, also. Natural selection can not be the governing cause in the origin of species, neither can chance (trial-and-error) in the meeting of germinal factors possibly produce species. If properties of mutual attraction and the laws of definite proportions and definite structural arrangement or grouping are essential in building the stable atom of an element and in the forming of chemical compounds, they must be even more necessary in the infinitely more complex organic world.

Take the illustration of the printer's pied type. If only two types, o and d, be thrown, the chances are equal for and against their falling in the order of d o, spelling a word. If a, b, l, n, and k be thrown the probability is that they will spell the word "blank" one time in 120 throws. If a, g, i, m, n, r, t and u be thrown, they probably will spell the word "maturing" only once in 40,320 throws. To spell two such eight-letter words in given sequence would require an average of nearly twenty-one trillion throws. If the whole alphabet, or indiscriminate parts of it, or a case of type be thrown, the chance of 16

contiguous letters spelling any two given eight-letter words is infinitely remote.

Then, after our words are formed through chance and they in turn are thrown, the chances against the forming of intelligent clauses, sentences and *expressions of thought* increase past any possibility of ever happening. Our letters which form words must have the property of mutual attraction that will cause them to assemble in orderly, definite arrangement, and the lack of such attraction for other letters that would spoil the words. Also, our words must assemble under some law of organization that compounds them in definite sequence into sentences and expressions of thought, before Hamlet is ready for the press.

I imagine that the alphabet of organic nature contains infinitely more letters than the English alphabet, and if a bird is of the order of a *thought*, then its expression must involve, in the evolution of the fundamental structure of the bird's organism, properties of mutual attraction between certain components, the absence of it among others, and the operation of laws of definite proportions and definite structural arrangement and grouping. This is consistent with what we are told is to be found within the cell.

Such properties and laws would precipitate some of the fog that clings around heredity and organic evolution. For example, the old conception of the origin of species (through variation and (or) mutations which are preserved or discarded through natural selection) demands a continuous gradation in the organic system, with no gaps between the species, which does not occur in nature. The links seem always missing. Others are: Why are some characters inherited and others not? Why are some inheritable characters transmitted in the Mendelian manner and some, it appears, otherwise?

Certain kinds of genes (or certain kinds of components of the gene) may have affinity or "valence" for certain other kinds of genes or their components with con-

genital valence (as may be illustrated by the sex chromosomes functioning in conjunction with like chromosomes of other cells) combining with them in definite proportions and varying grouping combinations having varying degrees of stability, just as we find among the electrons and protons in the atoms. Perhaps we should not carry this comparison too far so as to imagine revolution of certain components about certain other, nuclear components, yet the known properties and functions of the genes seem to demand that they be rather complexly organized. It may not be far wrong to think of the gene as a system of nuclear and orbital elements of opposite signs.

In organic *species*, then, we have aggregations of genes or their effective components whose mutual attraction and group pattern represent arrangements of maximum stability. In (some) *varieties* the arrangements are of varying degrees of instability with an inherent tendency to disintegrate. According to this hypothesis species are immutable in a like sense that the elements are immutable. They mutate in nature but can not, with our present knowledge and technique, be made to do so by artificial manipulation.

In *varieties* we may have three conditions. In the first and more rare, combinations and arrangements of the genes or their effective components that are in varying degrees of instability, with inherent tendencies to disintegrate, as in the case of a chemical analogue, thorium X. Such varieties may retain characters that appear specific for almost countless generations and may evolve into other pseudo species, but which ultimately revert (drop out factors) to more stable or truly specific combinations. This may account for instances of retrograding in the face of an almost general law of irreversibility, as in the case of three-toedness in the *Equus* and the *Rhea* and two-toedness in the *Struthio*.

The second class of varieties, which is a populous one, is comparable with chemical compounds. In this are the

hybrids. The compound sodium chloride is the end of its series. As such it will not chemically unite with any element or other compound. It is *sterile*. The same is generally true of hybrids. The chromosomes or genes, or their effective components, of two closely related species often mutually adapt themselves in new combinations resulting in hybrids, but these are "compounds," not "elements." When the sperm cell of the hybrid male unites with the ovum of the female there is only a *mixture*, not an organic union. The rare fertility found in hybrids may be due to one of the parents of the hybrids belonging to an "isotope" whose "atomic number" is nearer to that of the species of the other parent. If this be true, then the study of the germ and sperm cells of such parents should disclose in one of the parents chromosomal structure differing (isotopic) from the specific type. Just as some of the greatest mysteries of the atom were cleared up by Soddy in his study of the isotopes, so may a study of the parents of fertile hybrids clear up some of the dark places in the cell.

The third class of varieties is also large. Its members are the result of the organisms' response to their environment. To this may be likened the gaseous, liquid and solid, or the crystalline and amorphous states of the elements, or the conformation of crystals to the spaces in which they grow. A few examples are: climatic reaction upon pigmentation, variation in size and weight due to quantity and quality of nutrition, atrophy of organs and tissues through non-use and the development thereof through use. Characters of these varieties can never become specific in the organic system any more than the corresponding ones in the inorganic can become characters of an element.

A species and its immediate predecessor may present unexpected differences in both characters and chromosomal organizations. These differences may possibly be *periodic*. Thorium X does not disintegrate into the element of next lower atomic weight and number, but into

lead, which is separated from thorium by a whole period or octave in the scale of elements. When we explore further into the organizations of the chromosomes and genes, and then study these organizations in closely related species and isotopes within the species, we may find the explanation of why we never recognize the genesis of a species, either in contemporary life or in paleontology—why there is no connecting gradation between the species. There may never have been any connecting links.

It is an interesting coincidence that some of our paleontologists arrange animal life into eight super phyla, just as we have eight groups in the series of the elements. There are in each of these great phyla members which, in their evolutionary stagnation, resemble the inert gases—certain Foraminifera within the protozoa and the Blastozoa within the arthropoda, for example. And also within each species so exceedingly active in an evolutionary sense, or at least are so productive of varieties that they are comparable to the monads and dyads among the elements. Thus, if we find valency a property of the chromosome or gene organization, we will have another evidence of the ultimate simplicity of the universe, such as the comparison of the atomic and solar systems seems to show.

The organic series is not based upon the top of the inorganic series, else we should find uranium, thorium and radium composing organic matter instead of hydrogen, carbon, oxygen, etc. Organic evolution is not a continuation of inorganic evolution. In the face of their many similarities there is a fundamental difference between inorganic chemistry and biochemistry—between inorganic physics and biophysics. There is a discernible difference between inorganic and organic compounds, so there may prove to be "bio-hydrogen" and "bio-carbon," essentially different from their corresponding chemical elements. These bio-atoms may be so unstable as to break down under the most skilful technique on examination by

the chemist, or their distinguishing characters may be indiscernible with our present knowledge and methods.

The hydrogen spectrum indicates, under the quantum theory, that there are numerous kinds of hydrogen atoms, differing with the position and motion of a single electron, yet the different kinds of hydrogen are indistinguishable under any chemical examination. The investigations of Aston and Rutherford show that "the number of distinct species of atoms is much greater than was supposed."

Then why may there not be bio-atoms of hydrogen and carbon which, like other atoms, can not be caused to organize by any laboratory agency? Thus we might explain the long series of hydrocarbon compounds only a few of which can be directly produced in the laboratory, and do away with the absurd tenet that the multitude of life processes are *ordinary* chemical processes initiated through blind trial and error, while the intelligent direction of combined chemical wisdom has not thus far produced a single specimen of life, no matter how simple, in the laboratory.

Again, the lowest animal form is not in succession to the highest plant species. Animal evolution is not based upon the top of the plant series. While plants had to occur first, as a necessary condition for the existence of the animals, there is no reason to infer that animals ever evolved from plants. The logic of the conditions is that the elements, the plants and the animals arose from a common level—the more simple organizations of the atom, and represent three distinct phyla. We know that the mode of the evolution of the elements is in the increasing complexity of the organization of the atoms. The great virtues of simplicity and universal applicability attach to the hypothesis that the modes of plant and animal evolution are in the increasing complexity of two classes of bio-atoms whose sphere of activity is somewhere within the organization of the chromosomes, probably within the genes.

That the above outline contains inconsistencies and far-fetched comparisons is recognized, but all through it

there seem to be parallel threads of fact and truth that the conditions demand and without which they will not be satisfied.

Those who have read the above paper in manuscript and have been kind enough to criticize it are all alike in offering the objection that the gaps between species are by no means as common as implied in the paper. Most who read it, probably, will raise the same objection. Natural selection was so long accepted as the single mode of evolution that its necessary corollary, continuous gradation, seems to have become inseparable from the idea of evolution. Good modern authority on genetics recognizes species as natural groups with gaps between, *vide* Bateson in his address at Toronto, December, 1922, published in *Science* January 20, 1923.

The most exhaustive recent discussion of species and the species concept is by Hall and Clements in *The Phylogenetic Method in Taxonomy—the North American Species of Artemisia, Chrysothamnus and Atriplex*, a 1923 publication of the Carnegie Institution of Washington. After perhaps the most thorough study in the field that was ever given a similar group, and after presenting very fully all phases of their subject, they advance conclusions from which the following statement is compiled, mostly in their exact phrases, some of them several times repeated in their discussion. They do not undertake to "define" species, and this is only my attempt to embody their cardinal conclusions in a brief, only tentative definition: The best-approved concept of species, which is applicable to actual conditions in nature, is that species are definite phylogenetic units—natural groups—which show adequate morphological differentiation, *with definite limits, characterized as a rule by "actual gaps in evolution* (see page 15), which are gametically pure and breed true."

In discussing "divergence" and "convergence," they use this expression: "Thus, while *most species* will show

distinct gaps, it must be admitted that the gaps may be quite or completely closed in *some* instances, . . . ” The italics, of course, are mine, and it should also be stated that nothing is farther from these gentlemen’s minds than standing sponsor for such speculations as are presented by this paper, but their findings that in “*most*” cases there are distinct gaps between the species and that these gaps are “closed in *some* instances,” are facts for all purposes to be served.

In *Nature*, February 9, 1922, Willis and Yule give a summary of the results of their statistical studies which suggest what Pearl has referred to as “apparently a real law of organic evolution.” The conception can not be stated within the limits of this note, but it may be roughly illustrated—not by the old figure of ever-spreading branches of a tree, but by a more or less matured tree dropping a few seed, each of which contains the germ of a new tree which differs by *specific, and even generic* and larger *degrees* from the ancestral tree, and from the other offspring. By formulating from their paper the “apparent law,” we have something like the following: The velocity of evolution is uniform, *i.e.*, the *progress* of evolution, measured by the total number of species within any given group of ten genera, is directly proportionate to the *age*, measured by the area occupied by that group. They do not so state their conclusions, nor do they advance such conclusions except as highly probable interpretations of the conditions.

In *Science*, page 13, of July 2, 1920, David Starr Jordan calls attention to another “law” illustrated by the succession of two series of fishes and “other groups,” “showing apparently that non-specialization, ultra-specialization and loss of structures are all of secondary importance in the struggle for existence, and that they are conditioned on something else, a law not yet understood,” that this development is “pursued along what might seem to be a definite, determinative line.”

However divergent it may be from Jordan's general conception of evolution his tentative conclusion as regards these groups is that evolution pursues a definite, determinative line, in harmony with the theory of orthogenesis. With the, possibly unwarranted, assumption that these groups are representative of all others, and placing the conclusions just stated with the “apparent law” of Willis and Yule, we have the following interesting law (?): Evolution proceeds along a definite, determinative line, and at uniform velocity.

Should the existence of this law ever be proved, the survival of the fittest will remain no less a law, determining the shape and extent of each evolutionary tree, but not the time of fruiting nor the character of the fruit (mutations), except as it destroys the products of all new fruit which are not adapted to their environments.

A SECOND FACTOR FOR PRIMITIVE SPORO-PHYTE IN MAIZE¹

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IN an earlier paper (Eyster, W. H., 1924) are described maize sporophytes which do not become dormant in the seed stage, but have a continuous development from the fertilized egg. Since they resemble in this respect the sporophytes of more primitive plants, as the Bryophytes and Pteridophytes, they were designated as *primitive sporophytes*. Before the ears which bear the viviparous sporophytes are mature, the embryos burst the pericarp of the kernels and proceed to develop into seedlings, in a way quite like the sporophytes on the strobili of *Selaginella rupestris*. Growth continues so long as there is sufficient moisture in the endosperm and cob to permit it. When the water supply fails, the seedlings wilt and die. If the living seedlings are transferred from the maturing ear to moist filter paper, or planted in soil, growth continues normally. Because of the absence of chlorophyll these precocious seedlings died when the food supply in the endosperm was exhausted. This viviparous condition in maize was found to be inherited as a simple Mendelian recessive and is the expression of a factor which was designated *pm*. The factor pair *Pm pm* is closely linked with a factor pair for yellow endosperm and very closely or completely linked with a factor pair for albinism.

Similar sporophyte characters in maize have been found by others. At the Connecticut Station, Jones (Mangelsdorf, P. C., 1923) found germinating kernels on ears of two strains of maize which are thought to be in-

¹ The investigation reported in this paper was made in connection with genetic studies of maize in the department of Field Crops, Missouri Agricultural Experiment Station.

herited. Lindstrom (1923) described an ear of maize with kernels that had germinated prematurely and died. On this ear there were 149 non-defective and 44 defective kernels. Of the non-defective kernels 112 had dormant embryos, while 37 had embryos that had failed to become dormant in the seed stage.

The present paper is concerned with continuously growing sporophytes that occurred in a strain of maize quite distinct from that in which were found the sprouted kernels previously described (Eyster, W. H., 1924). Inter crosses have shown that the sprouted kernels in the two strains are due to distinct factors. In order to distinguish between these apparently identical but genetically distinct characters they will be called *primitive*₁ and *primitive*₂, respectively, with the factorial symbols *pm*₁ and *pm*₂.

INHERITANCE OF PRIMITIVE₂ SPOROPHYTE

The F₁ kernels from the cross, *normal* × *primitive*₂, have dormant embryos. When F₁ plants are self-fertilized ears are produced which bear kernels with dormant and growing embryos in the relation of 3 to 1, as shown by the data in Table I. Of 881 kernels from two segre-

TABLE I

F₂ KERNELS FROM THE CROSS NORMAL × PRIMITIVE₂

Pedigree	Dormant embryos	Growing embryos	Total
5025—2	350	120	470
5025—7	304	107	411
Totals, observed	654	227	881
Expected (3:1)	661	220	881
Deviation	— 7	+ 7	

gating ears 654 had normal and 227 growing sporophytes. This is a deviation of 7 ± 8.67 from the numbers expected for a 3:1 ratio.

INDEPENDENCE OF THE FACTORS *pm*₁ AND *pm*₂

Mention has already been made of the fact that crosses between heterozygous plants of the two strains in which germinating kernels have been found give kernels with

dormant embryos. Apparently, the primitive sporophyte condition of the two strains is due to different factors. The F_1 plants from the cross $Pm_1 pm_1 \times Pm_2 pm_2$ should be of the following genotypes:

Genotypes of F_1 Plants	Expected Results on Ears of Self-fertilized F_1 Plants	
	<i>Dormant Embryos</i>	<i>Growing Embryos</i>
$Pm_1 Pm_1 Pm_2 Pm_2$	all	.
$Pm_1 pm_1 Pm_2 Pm_2$	3	1
$Pm_1 Pm_1 Pm_2 pm_2$	3	1
$Pm_1 pm_1 Pm_2 pm_2$	9	7

One fourth of the self-fertilized F_1 plants should produce ears with all kernels normal, one half of them should have ears with normal and sprouted kernels in the relation of 3:1, and one fourth of them should produce ears with normal and sprouted kernels in the ratio of 9:7. These results have been realized. Of the six F_1 plants that were tested, two had ears with normal kernels only, four had ears with normal and sprouted kernels in the ratio of 3:1, and two had ears with normal and sprouted kernels in the relation of 9:7. The numbers are small, but are in exact agreement with expectation. Ears with normal and sprouted kernels in the ratio of 9:7 are conclusive evidence that there are two factors for primitive sporophyte in maize. In Table II are given the results

TABLE II

F_2 KERNELS FROM THE CROSS PRIMITIVE ₁ \times PRIMITIVE ₂			
Pedigree	Dormant embryos	Growing embryos	Total
5025—3	239	202	441
5025—13	303	224	527
Totals, observed	542	426	968
Expected (9:7)	544.5	423.5	968
Deviation	— 2.5	+ 2.5	

from two ears which segregated primitive sporophyte₁ and primitive sporophyte₂. There is a close agreement between the observed and expected numbers, with a deviation of only 2.4 ± 10.41 .

SUMMARY

Maize embryos normally become dormant in the seed stage and remain so until conditions favorable for growth are provided. Kernels have been found with embryos that do not become dormant in the seed stage, but continue to grow as long as there is enough moisture in the endosperm and cob to permit it. This growth characteristic has been called *primitive sporophyte* because of its resemblance to the sporophytes of more primitive plants. Primitive sporophyte is inherited. There are two independent factors for the primitive sporophyte condition in maize, pm_1 and pm_2 .

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THE DISTRIBUTION OF CHROMOSOMES IN THE POLLEN-GRAINS OF A TRIPLOID HYACINTH

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IN some monocotyledons with long chromosomes (*Cypripedium*, *Narcissus*, *Hyacinthus*, etc.), the first division in the pollen-grain, which separates the generative and vegetative nuclei, affords, at the metaphase, a better opportunity for accurately counting and comparing the chromosomes than either the first or the second metaphase in the pollen-mother-cells. This division in the pollen-grain is also perhaps sometimes a better stage at which to count and study the chromosomes than the metaphase in the root-tips; because there are only half as many chromosomes, and the young pollen-grains can probably be more rapidly fixed.

Bulbs of hyacinth (*H. orientalis*), towards the end of the year, were put in a warm room over water; one side of each bulb having been cut away, so that the young raceme was visible. At intervals, a bud was removed, and the contents of the anthers pressed out in strong iron-acetocarmine (1). When the right stages were found, preparations were made, in which the amount of liquid was so adjusted that the capillary pressure of the large coverglass somewhat flattened the young pollen-grains. For the counting and classification of the chromosomes a water-immersion objective was found best, used with yellow-green light (Wratten light filters, 57A or 58), and an immersed achromatic condenser (2).

As a representative of the presumably diploid hyacinths, bulbs were obtained under the name "Yellow-hammer." They had no purple on the bulb-scales, and those that flowered bore sulphur-yellow flowers. (A clone of this name had been found by de Mol (6) to have 16 chromosomes in the root-tips; 8 long, 4 medium and 4

short.) In the pollen-grains, the metaphase showed 8 chromosomes. There were three or perhaps four kinds. Four were long and V-shaped, of approximately equal size, each with a constriction at the center. This constriction often showed as a narrow clear space extending completely across the chromosome. (One or two of the 4 V's not infrequently showed a second constriction; but it could not be determined whether this subterminal constriction was a characteristic of only one pair, or of both pairs of V's, or was accidental.) The two medium chromosomes were apparently identical in size and shape. They often had the form of J's, and a subterminal constriction was present in both. The two short chromosomes appeared similar to one another, and usually showed a subterminal constriction. (These constrictions were not figured by de Mol (6) in his preparations of the root-tips.) Thus, in this presumed haploid group of chromosomes in the pollen-grain, we meet apparently a number of sets of two similar chromosomes each; which is the state of affairs to be found in the pollen of tetraploid plants (4). Hence this clone of hyacinth is possibly either tetraploid, or still shows the signs of descent from tetraploid ancestors. The wild form of *H. orientalis* has not yet, so far as I know, had its chromosomes counted.

As a sample clone of those found by de Mol (6) to have 12 large, 6 medium and 6 small chromosomes in the root-tips, and presumed to be triploid, bulbs were obtained under the name "Lady Derby." These had purple bulb scales, and the ones that flowered bore pink flowers. In the metaphase of the first division in the pollen-grains, the numbers of accurately counted and classified chromosomes ranged from 8 to 14, though some other cells had over 14. The one cell which had 8 chromosomes had the same size formula, $4L + 2M + 2S$, as did all the pollen-grains of the clone "Yellow-hammer" whose chromosomes were counted.

If this clone ("Lady Derby") is a triploid, or double triploid, 8 of the chromosomes in the pollen-grain (namely, $4L + 2M + 2S$) will have probably come from

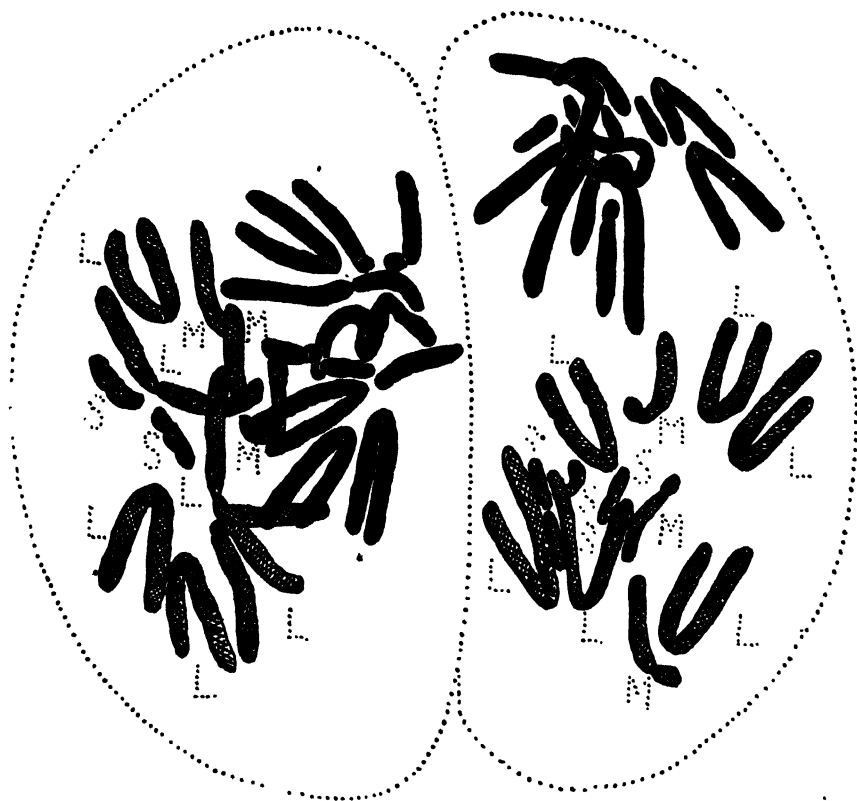


FIG. 1. Divided pollen-mother-cell of the triploid hyacinth, Lady Derby, at the anaphase of the second division. In the left-hand half, the two groups have each 6 long, 3 medium and 2 short chromosomes. In the right-hand half the two groups have apparently 6 long, 3 medium and 4 short chromosomes. The total is 12 long, 6 medium and 6 short chromosomes. The same numbers were found by De Mol in the root-tips.

pairing with 8 similar chromosomes at the first metaphase in the pollen-mother-cell. The remaining 8 chromosomes of the pollen-mother-cell would probably be distributed according to the laws of chance to the two nuclei resulting from the reduction division (3), and hence to the two pairs of pollen-grains which come from each pollen-mother-cell.

The chromosomes were counted and classified at the metaphase in 52 pollen-grains. Many other pollen-grains showed the same stage, but the chromosomes overlapped so that there would have been some uncertainty in their classification. Since, other things being equal, the less

the number of chromosomes the more widely they were spaced at the metaphase, a few more pollen-grains with a number of chromosomes below the mean (12 chromosomes) would probably be included in the number counted than would have come in a random sample of the pollen-grains. However, if the distribution of the extra chromosomes was a random one, this source of error should not obscure the results, if duly allowed for.

After subtracting $4L + 2M + 2S$ from the number of chromosomes found in each pollen-grain, the resulting excesses of the numbers of chromosomes over eight are given in Table I.

TABLE I
DISTRIBUTION OF EXTRA CHROMOSOMES IN 52 POLLEN-GRAINS OF A
TRIPLOID (OR DOUBLE TRIPLOID) HYACINTH

No. of extra chromosomes	0	1	2	3	4	5	6	7	8
No. of pollen-grains	1	5	3	15	11	14	3
<i>Calculated</i>	0.2	2	6	11	14	11	6	2	0.2

Table I shows that out of the 52 pollen-grains there were 7 more grains below the middle class (with 4 extra chromosomes) than there were above this class. The two lowest classes include 6 pollen-grains, and the two highest classes have none. A change of 3 pollen-grains from low to high would about balance this, and the total number of extra chromosomes would be increased by 18 or 19.

TABLE II
DISTRIBUTION OF THE EXTRA CHROMOSOMES IN THE THREE SIZE
CLASSES IN 52 POLLEN-GRAINS

	Long	Medium	Short	Totals
Numbers of chromosomes	97	51	40	188
<i>Calculated</i>	104	52	52	208
Deviations	— 7	— 1	— 12	— 20

Table II shows that while a chance distribution ordains that about 208 extra chromosomes would probably be found in the 52 pollen-grains of the triploid plant, actually only 188 extra chromosomes were counted, there being 20 chromosomes lacking. Thus the data in Tables I and II agree fairly closely with the results expected

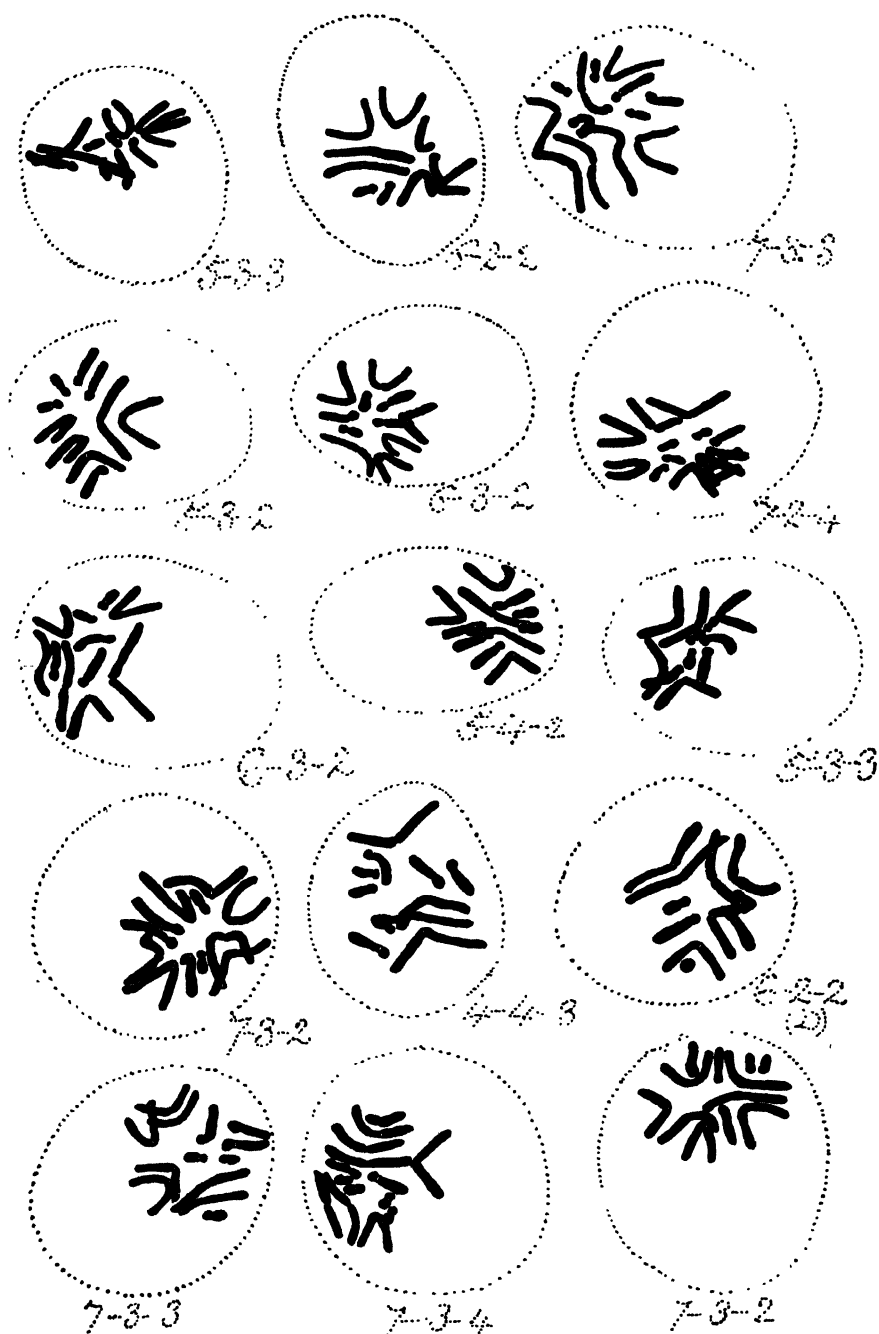


FIG. 2. Fifteen pollen-grains from the triploid clone, Lady Derby. These were taken in sequence from one bud, and were all that showed clear, readily countable, chromosome plates. The longitudinal split of the chromosomes is not shown in these camera drawings, though obvious in the preparation. The numbers of long, medium and short chromosomes are given. In the twelfth pollen-grain, there was a small spherical detached chromosome, which was not in the metaphase plate. The figure was drawn under the 8 mm apochromatic, with yellow-green light.

from a random distribution of the 8 extra chromosomes, except for a small deficit of cells with more than 5 extra chromosomes.

TABLE III

DISTRIBUTION OF THE LONG EXTRA CHROMOSOMES IN 52 POLLEN-GRAINS

Extra Chromosomes	4 long	3 long	2 long	1 long	0 long
Numbers of Pollen-grains	3	13	18	10	8
<i>Calculated</i>	3	13	20	13	3

Table III shows the frequencies of the different combinations of long extra chromosomes. The proportions for random distribution are given by the terms of the binomial $(1 + 1)^4$, and the fit is fairly close, there being however an excess of 5 cells without any long extra chromosomes.

TABLE IV

DISTRIBUTION OF THE MEDIUM EXTRA CHROMOSOMES IN 52 POLLEN-GRAINS

Extra Chromosomes	2 medium	1 medium	0 medium
Numbers of Pollen-grains	13	25	14
<i>Calculated</i>	13	26	13

Table IV shows the frequencies of the different numbers of medium extra chromosomes. The figures fit closely to the chance results as calculated.

TABLE V

DISTRIBUTION OF THE SHORT EXTRA CHROMOSOMES IN 52 POLLEN-GRAINS

Extra Chromosomes	2 short	1 short	0 short
Numbers of Pollen-grains	10	20	22
<i>Calculated</i>	13	26	13

Table V gives the results for the short chromosomes, which show a deviation of 9 zeros in excess, or over 4 times the probable deviation, doubtless partly for the reason given above.

Hence the agreement with the results of random distribution for all three classes of chromosomes is close, if we allow for necessary selection in counting slightly favoring the cells with the smaller numbers of chromosomes.

Summary: (1) The pollen-grains of a diploid (or double diploid) clone of hyacinth showed two pairs of long V-shaped chromosomes with a median constriction; one pair of shorter J-shaped chromosomes with a subterminal constriction; and one pair of short chromosomes, also with a subterminal constriction. The presence of a number of pairs of chromosomes, the members of each pair being apparently identical, is characteristic of the pollen of tetraploid plants.

(2) The pollen-grains of a triploid (or double triploid) clone of hyacinth showed a number of chromosomes ranging from 8 to over 14. These belonged to the same size classes as those of the former clone.

(3) The extra chromosomes in the pollen of the triploid hyacinth which were in excess of the numbers in the pollen-grains of the diploid hyacinth, were distributed, collectively and in each size class, according to the laws of chance, after allowing for a source of slight error.

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THE PROBLEM OF INCIDENCE IN COLOR BLINDNESS

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COLOR blindness is the best known and most often cited form of sex-linked inheritance in man. Text-book statements and most special articles relating to the genetics of this trait tend to convey the impression that the observed distribution of cases is in complete accord with theoretical expectations based on the assumption that the heredity of color blindness in man is similar to that of white eye color in *Drosophila*. A survey of the literature,¹ however, shows that, wholly apart from questions of diagnosis and interpretation, some possible exceptions to the rule have been reported and that the total amount of critical data on record is rather inadequate. Some, or all, of the doubtful cases may be due to incomplete or inaccurate records but, however that may be, there is a clearly demonstrable lack of agreement between the number of color-blind women recorded statistically and the number to be expected according to the current theory. This invests the problem of the inheritance of color blindness with a new interest and should make a detailed study of the subject profitable from the point of view of both human genetics and demography.

It may be recalled that a sex-linked trait, such as color blindness is presumed to be, is generally thought of as conditioned by a recessive gene located in the sex chro-

¹ Recent digests of the literature on the heredity of color blindness may be found in the papers by A. Vogt and R. Klainguti (Weiterer Untersuchung über die Entstehung der Rotgrünblindheit beim Weibe, *Archiv für Rassen- und Gesellschaftsbiologie*, 14: 129-140, 1922), Gustav Döderlein (Über die Vererbung von Farbensinnstörung, *Archiv für Augenheilkunde*, 90: 43-46, 1922), and Ingolf Schiötz (Colour blind females: The inheritance of colour blindness in man, *British Journal of Ophthalmology*, 4: 345-359, 393-403, 1920).

mosome. Consequently, every male zygote which receives this gene in its single sex chromosome develops the trait, while only such female zygotes as receive the gene in both sex chromosomes actually develop it. Those female zygotes which have the gene for color blindness in only one chromosome, and are consequently heterozygous, develop into women with normal vision who are nevertheless capable of transmitting the trait. Such women are commonly designated as "conductors." As a result of these relations it is readily seen that a color-blind man, since he receives his sex chromosome from his mother, must always be the son of a woman who is either color blind or a conductor, and that such a woman will produce color-blind sons irrespective of whether or not her husband is color blind. The mothers of color-blind women are genetically equivalent to the mothers of color-blind men, but they are able to produce color-blind daughters only by virtue of being married to color-blind husbands—since a female to be color blind must receive genes for the trait from both of her parents. Consequently, the histories of color-blind females should furnish by far the most critical data bearing on the heredity of the trait. This has been clearly recognized by several investigators and yet, despite the fact that one woman in every 250 is said to be color blind, the number of records which are sufficiently complete is not very great.

When the prediction of the geneticist is checked against the figures of the statistician it is again the data on color-blind females that constitute the most important material, since it is the frequency of this class which gives the most delicate index of the agreement between the results obtained by the two methods. The statistical data are extensive and have been collected in a number of countries and over many years. The results are rather consistent,² whether the figures are compiled in Central Europe, Scandinavia or elsewhere, and generally agree in showing that about 4 per cent. of men and .4 per cent. of

² Cf. Schiötz, *loc. cit.*, and also Vogt and Klainuti.

women are color blind. The prevailing ratio of ten color-blind men to one color-blind woman has often been remarked upon. We may now inquire whether these are the proportions that should be expected in the case of a simple sex-linked trait.

If we let C represent the gene for normal color vision and c that for color blindness we find that in the populations studied statistically there are 96 men of the genetic constitution C — to every 4 of the constitution c —. Since each male has only one or the other of these genes but not both, the numerical ratio between the two types of genes in the male germ plasm of the whole population is as 96 to 4. Statistics extending back for the past half century indicate that this ratio has been maintained rather constantly. Under the circumstances it is to be expected that the numerical ratio between the two genes is essentially the same in the female part of the population, since any change of equilibrium in one sex would be followed in the next generation by a corresponding change in the other sex.³ But since the female zygote has two sex chromosomes there are three possible combinations of C and c , and in the absence of selective mating in reference to color blindness these combinations should have a random distribution represented by the algebraic square of 96 and 4 or 92.16 per cent. CC , 7.68 per cent. Cc and .16 per cent. cc . Such a population would always produce 4 per cent. of color-blind males but only .16 per cent. of color-blind females. This is the expectation if color blindness is due to a simple sex-linked trait and the statistically determined 4 per cent. of color-blind males is correct. Any appreciable deviation from it calls for an explanation. If it were assumed that the statistically reported .4 per cent. is correct for color-blind females then it might be expected that there would be about 6.3 per cent. of men who show color blindness,

³ Various phases of this question as it relates to animal and plant breeding have been discussed by Jennings, Robbins, Wright, Wentworth and Remick in *Genetics* from 1916 on.

which is over 50 per cent. greater than the observed number. Since color-blind men are ten times as numerous as color-blind women it is probable that the data for men is, if anything, more accurate than that for women and it may consequently be stated that color-blind women are found to be about two and a half times as frequent as the theory of heredity and random mating would lead one to expect (*i.e.*, .40 per cent. instead of .16 per cent.).

Apparently the only writer on the subject who has fully appreciated the mathematical requirements of the theory is Schiötz who by another method, arrives at the same figures as indicated above. Schiötz, however, found in a series of 2,200 girls and 2,005 men 20 girls and 202 men who had *more or less* defective color vision. The numbers who showed typical red-green blindness were not essentially different from those observed by other students; nevertheless, Schiötz prefers to group all those with defective color vision in one class, especially since by doing so he arrives at the only pair of values (approximately 10 per cent. of the males and 1 per cent. of the females) which should theoretically remain stable in a 10 to 1 ratio. He states⁴ that "from a purely mathematical point of view such a set of values . . . should remain constant for an indefinite future, and it is probable that it has existed for a considerable period" and further that ". . . we may be justified in drawing the conclusion that the values obtained by the collective statistics of 1870-80, *viz.*, 3.94 per cent. colour-blind men and 0.33 per cent. colour-blind women, may be explained through insufficient testing methods, since such relative values could not possibly exist in any population, still less remain stationary. . . . On the other hand, the relative proportion between colour-blind men and women, as given in the old statistics, *viz.*, 1:10, or a little more, allows the conclusion that colour blindness actually occurred to the same extent at that time that it does now."

Schiötz's proposed solution of the problem involves the difficulty that the class of individuals who are to be

⁴ *Loc. cit.*, p. 358 and ff.

counted as color blind (deuteranopes or "deuteranomalous") is greatly increased and that about 60 per cent. of subjects require a very special technique for their detection. In itself this involves no real improbability, but the fact that most determinations in the past have been based on the cruder methods of diagnosis would lead one to expect that geneticists would have noted either that various grades of color blindness are due to several cumulative factors, or that color-blind women are more often than not the daughters of parents who could not be recognized by ordinary means as other than normal. The former alternative is generally considered improbable (Vogt and Klainguti). As to the latter, since it is scarcely to be expected that greater skill will be exercised in diagnosing the condition of the parent than of the child, the technique which indicates that only 0.4 instead of 1.0 per cent. of women are color blind should also seem to show that 60 per cent. of color-blind women have normal fathers.⁵ Further, color-blind parents should produce children of both sexes who could not be distinguished from those with normal vision. But this is far

⁵ Since among 1,000 men there should be 40 subjects who would be universally recognized as color blind and an additional 60 who would ordinarily pass as normal but whom Schiötz would put in the color-blind class, and among the same number of women 4 and 6 subjects in these respective groups, the probable frequency of expected types of marriages and the resulting offspring can be computed. In the following table these two classes of subjects are designated as color blind and "normal."

MARRIAGES PER 1,000,000 CAPABLE OF PRODUCING COLOR-BLIND DAUGHTERS
AND THE EXPECTED DISTRIBUTION OF COLOR-BLIND AND NORMAL GIRLS

Parental characters		Frequency of mating	Relative number of daughters		
Mother	Father		Color blind	“Normal”	
Color blind	Color blind	$4 \times 40 =$	160	64	96
Color blind	“Normal”	$4 \times 60 =$	240	96	144
“Normal”	Color blind	$6 \times 40 =$	240	96	144
“Normal”	“Normal”	$6 \times 60 =$	360	144	216
Conductor	Color blind	$180 \times 40 =$	7200	1440	2160
Conductor	“Normal”	$180 \times 60 =$	10800	2160	3240
			Total	<u>4000</u>	<u>6000</u>

Of the 4,000 color-blind daughters, 2,400 (*i.e.*, $96 + 144 + 2160$), or 60 per cent., should be expected, on the hypothesis being tested, to have normal fathers.

from what is indicated by such records as are available. If the theory of multiple factors were to be invoked the 10:1 ratio would no longer hold, so until this dilemma can be explained the suggestion of Schiötz can not be accepted as wholly satisfactory.

Several other suggestions as to the cause of the discrepancy between the observed and expected number of color-blind women may be mentioned, although in the absence of better data none of them can be regarded as more than a possibility. It may be, for example, that not all cases of color blindness are hereditary. If from generation to generation about 0.26 per cent. of all men and an equal number of women became color blind from other than hereditary causes the absolute and the relative incidences would remain constant over a long period. The objections to this idea are essentially the same as those to the theory of Schiötz. Either view is easily susceptible of critical testing.

Another possibility, somewhat less easily eliminated, is that not all hereditary color blindness is sex linked. If the above mentioned 0.26 per cent. of individuals had a distinct and non-sex-linked form of hereditary color blindness the conditions would be met statistically and would be rather difficult to analyze genetically. But even in this case a large percentage of color-blind women should be found to have normal fathers.

The presence of a lethal factor associated with sex-linked traits has been suggested by Little and Gibbons.⁶ A lethal factor closely linked with that for color blindness and occurring with the proper frequency (about 0.52 per cent.) might result in the production of cases of color blindness with the observed distribution, but only for one or two generations, since a sex-linked lethal, even if it appeared in any appreciable frequency, would rapidly run out in the absence of very frequent mutations

⁶ Little, C. C., and Gibbons, M. Evidence for sex-linked lethal factors in man. *Proceedings Society of Experimental Biology and Medicine*, 18: 111-115. 1920-1921.

(about 1:400 in this case). So it would seem that this possibility can not be invoked as an explanation.

In contradistinction to the suggestions that have been presented, which assume either error of diagnosis or the presence of unanalyzed genetic factors, it may be that the biasing influence is of an entirely different character and belongs to a group that haunts some of the difficult places in the field of demography. In this connection the effect of differential marriage selection may be considered. That individuals of either sex are either preferred or rejected on the basis of the quality of their color vision is highly improbable, but it is possible that some factor of custom or locality may be of importance. It might at first seem significant that in all communities there is for each individual a considerable group of the opposite sex with whom marriage is "taboo." This group always includes sibs, and generally many others. Since color-blind men are the potential fathers of color-blind women and since the sisters and cousins of color-blind men include a larger proportion of conductors than do women taken at random, the chances of a color-blind man marrying a woman who is a conductor are somewhat diminished below what they would be if matings were really "random." This tends to reduce the number of color-blind women below what would be expected from strictly random mating. Under almost all circumstances the effect would be insignificant, and in every case it would tend to decrease the number of cases of color blindness in women, while the problem before us is to explain an excess number of such cases.

The alternative possibility, namely, that marriages as actually contracted generally involve a greater amount of inbreeding than would "random mating," affords the ground for a more promising suggestion. Close inbreeding would tend to increase the number of females showing a recessive sex-linked trait since the incidence of conductors is relatively high among the near relatives of color blind men. With the closest form of inbreeding, the

incidence of color blindness in women would approach the incidence observed in men through the gradual elimination of the class of heterozygotes (Cc). In the male sex, where there is no such class, the incidence remains the same irrespective of types of mating. Consequently, if the observed incidence in the male is 4 per cent., the hypothetical maximum incidence in the female would also be 4 per cent. But if matings were wholly at random the incidence in the female would be .16 per cent., or if the absence of close inmarriage is taken into account not more than 3 per cent. less than this (or .155 per cent.), depending on the average number of children to the family and other incidental factors, which in the aggregate are insignificant. It would have been expected, therefore, that the number of color-blind women would fall between the limits 0.155 per cent. and 4.000 per cent. The observed incidence, 0.400, is in accord with this expectation and indicates that there must be some inbreeding over and above what would occur if matings were random except where two sibs are concerned. The range between 0.155 and 4.000 is 3.845 and the observed .4 represents an elevation of about 6 per cent. of this value. This, then, might be taken as an index of inbreeding for the whole population. In other words, if the suggestion holds, the sex ratio in color blindness furnishes one of the simplest and perhaps most accurate indications of the amount of inbreeding in a population.

In considering this criterion of inbreeding there is a subsidiary possibility to be taken into account. Large populations are generally divisible into small natural groups ultimately descended from relatively few ancestors. The characteristics of such groups will depend primarily on the characteristics of the original germ-plasms from which the groups are derived, and these germplasms will be likely to differ from each other in various respects. Such a trait as color blindness might be present in some of the ancestral stocks, absent in others, so we may easily imagine a population in which

there are socially or geographically isolated groups, some characterized by a relatively high incidence of color blindness, others by a low incidence. The occurrence of local groups with their own peculiar incidence of the trait is suggested by the often quoted statements of Cohn and Magnus as to the relatively high frequency of color blindness among the Jews of certain localities. In such groups the frequency of color blindness in women would depend on the character of the original stock and the amount of inbreeding occasioned by barriers of various sorts. If the incidence of color-blind females in any group is designated, in terms of per cent., as m , that for males in the same group should be $100 \sqrt{m}$, as is apparent from the theory of sex linkage and as Schiötz has further emphasized. Consequently, the ratio of ten color-blind men to one color-blind woman may be significant since, as Schiötz rightly insists, the only population which could show this ratio in the presence of random mating is one in which 10 per cent. of the men and 1 per cent. of the women are color blind. But reasons have already been given for thinking that this is not, as Schiötz maintains, the condition in the population as a whole.

If, however, we assume a population so constituted that on the average two of the self-limited (or "endogamous") groups out of five are characterized by the presence of color blindness in 10 per cent. of their men and 1 per cent. of their women, while in the other three groups color blindness is practically lacking the expectations based on genetics will agree with the results obtained statistically (since 10 per cent. of 2 is 4 per cent. of 5, and 1 per cent. of 2 is 0.4 per cent. of 5). In other words, as a hypothesis, the suggestion of subordinate groups showing differential frequencies of color blindness is adequate to account for the observed facts. If such groups do in reality exist and are of any considerable size, marriages within them might be wholly free from what is ordinarily classed as consanguinity without reducing appreciably the relative number of color-blind females. In other

words, the statistical test of inbreeding suggested above does not differentiate between the effects of a few closely consanguineous marriages and a larger number that are not so close, it may indicate the presence of inbreeding but not its exact nature. Particularly interesting in this connection would be an adequate comparison of the frequency of color-blind women in two countries, one with much, the other with little intermingling of peoples, and especially so if it should be found that the frequency of color-blind men was the same in both places.

To summarize briefly: (1) The available data do not suffice to show conclusively that color blindness is a simple sex-linked trait, although they make it appear probable that such is the case; (2) the number of color-blind females is in excess of expectations based on the theory of sex-linkage and random mating; (3) several hypotheses which might account for this discrepancy are susceptible of adequate testing through the accumulation of critical data; (4) if, as seems likely, the current views as to the genetics of color blindness prove correct, the distribution of this and other sex-linked traits may afford the means of establishing a useful index of the amount of inbreeding within demographic units.

DEPRESSING EFFECTS OF STRYCHNIN ON THE VORTICELLA AND OTHER UNICELLULAR FORMS

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It is definitely known that strychnin acts selectively on the spinal cord of the vertebrate and makes the animal first more and then less responsive to peripheral stimulation than it normally is. Of the many hypotheses which may be formed to explain the heightened irritability and the subsequent depression, one of the most probable ones seems to be that the synaptic connections in the cord are made first better and then worse than usual. When the connections are good, as in the earlier stages of poisoning, the impulses arising from peripheral stimulation pass through the cord and reach muscles of all kinds in greater showers, and later, during the state of depression or narcosis, the synaptic connections are so poor that the impulses are blocked in the cord. Experimental results¹ which seem to support this view is that some peripheral nerves, when excised and exposed to ether or chloroform vapors, first elongate slightly and then shorten, becoming eventually somewhat shorter than they were normally. If the foreign substance, strychnin, causes the nervous elements of the cord to change in a similar way, the synaptic connections there should be better at the time that the elements are elongated and poorer when the same elements are later abnormally short. This would account very nicely for the initial state of hyperexcitability and also for the later state of depression or narcosis of the animal as a whole.

To be contrasted with the system of thinking just presented is the view of some that strychnin merely stimu-

¹ Swindle, P. F., "Quantum Reactions and Associations," p. 298, 1922.

lates the motor elements, of some that it stimulates the sensory elements, and of some others that it stimulates both the motor and sensory elements of the cord. In any of these cases the further assumption is that the nervous elements which are rendered unusually irritable by the strychnin respond now more readily and in greater numbers when impulses reach them from the periphery. Then, of course, the same elements must become less irritable later on to cause the state of narcosis.

In view of the circumstance that it is not possible to actually observe synaptic changes or to observe directly any change in irritability of the nervous elements of the cord, we chose to determine the effects of strychnin on some single cells whose relative irritability under various conditions can be determined with a reasonable degree of certainty. If strychnin should make any of these cells, such as the vorticella, more responsive to external stimuli the theory of stimulation of the nerve cells would seem to be the more useful explanation for the strychnin effects in the vertebrate, but if strychnin should cause no increase in the irritability the theory of the synaptic change would seem to be the more probable one.

The results of the present investigation show that strychnin does not render the unicellular forms more irritable to stimuli, and the view that strychnin can make a cell of the cord abnormally irritable is accordingly discredited by our results. Our data are opposed to those obtained by many other investigators who believed they found that the unicellular forms react to strychnin similarly as the higher animals do. (The probable reasons for the discrepancies will be pointed out from time to time in the body of the paper). In his review of the work of others in this field Poulsson² states that the greater number of the descriptions concerning the reactions of the infusorians, etc., to strychnin show that these forms react to the drug in essentially the same way as higher

² Poulsson, E., *Handbuch der Experimentellen Pharmakologie*, 2. Bd., S. 327, 1920.

animals do. He reminds us that if the solution is sufficiently dilute there soon occurs a state of hyperexcitability and spasticity and later depression and death.

If it is true that the unicellular forms react to strychnin in essentially the same way as the higher animals do, we should be able to so regulate the concentration of the drug that we can observe first a state of heightened responsiveness to external stimuli, then spasms, then depression, and later on (if the drug is removed gradually) a second series of spasms. The second series of convulsions can be obtained with the frog as it excretes the strychnin, because it breathes to a certain extent through its skin and can therefore live after the strychnin has depressed the respiratory movements. The second series of spasms can be obtained with the dog also, if artificial respiration is given.

ABSENCE OF SECOND STATE OF HYPEREXCITABILITY OF STALK OF VORTICELLA

We were able to observe neither an increased responsiveness of the stalk nor a spasm of any sort when the strychnin was removed in time for the animals to survive and approach their normal state of activity. The strychnin was removed by washing the animals with water which flowed underneath the cover-glass. The water was applied at one edge of the cover-glass and was absorbed with filter paper at the opposite side. Not only were the reviving and revived animals less irritable to external stimulation, but the number of spontaneous contractions of the stalk was far below the normal number. We have not been able to learn from the literature that any of the earlier observers attempted to look for a second series of spasms.

Since depression only and no indication of a state of heightened activity in the revived animals was seen, our observations were later limited to the effects of strychnin between the time of application of the drug and the death of the animals. An attempt was made to determine

whether or not there was an increase in the number of spontaneous contractions of the stalk and whether or not the animals became exceptionally irritable to external stimuli, such as jarring the table or the microscope.

ABSENCE OF INITIAL INCREASE IN IRRITABILITY OF STALK OF VORTICELLA

In order to avoid mechanical stimulation, which might be produced by adding strychnin in solution to the water containing the animals, we placed crystals of strychnin sulphate either in the uncovered water or in the water at the edges of the cover-glass. The process of adding the crystals might disturb the animals, but this initial disturbance could not interfere with the observations because the crystals dissolve so slowly that the first effects of poisoning occur long after the crystals are applied. It is quite different when solutions are used, because the poisoning occurs so quickly that the increased activity of the animals due to the mechanical stimulation produced by adding the solution might be confused with postulated effects due to the strychnin poisoning. For instance, if the solution is applied at one edge of a cover-glass, a current of considerable duration is set up in the water and causes the animals to be unusually active for some time. It is our belief that some of the earlier observers probably mistook the effects of this mechanical stimulation for an initial effect of strychnin as a poison.

When a minute crystal of strychnin is placed carefully into a small volume of water containing some vorticellae there is no initial increase attributable to strychnin in the irritability of the stalk to external stimuli, and there is also no increase in the number of spontaneous contractions of the stalk. The animals finally die, however, of strychnin poisoning. The vorticella is therefore quite unlike the frog or the dog with respect to its reactions to strychnin. In the case of the frog, the gradual application of strychnin to the water causes a state of increased responsiveness of the animal. As more and more strychnin

nin is added spasms eventually occur. Both the frog and the vorticella are poisoned when strychnin is added gradually to the water, but one of these animals becomes more irritable to external stimuli, while the other one suffers depression instead.

In attempting to determine whether or not the strychnin caused the vorticellae to become unusually irritable to such a mechanical stimulus as jarring the table or the microscope, one special difficulty was encountered. If a normal animal was jarred as frequently as once every ten seconds it usually ceased responding to the stimulus before ten minutes passed. In order to determine the normal irritability of the animal to the jarring it could have been stimulated a small number of times in a smaller number of minutes, but even in one minute there was frequently a decrease in the irritability when only three or four stimuli were presented. The fact that the unpoisoned animals became less irritable to the jarring is important. It shows that if they were stimulated for ten minutes or for one minute to obtain the normal state of irritability they were less irritable when the strychnin was applied. It shows, also, that if one should not obtain the normal and begin jarring the animal immediately after the drug is added, one would observe at first a much greater responsiveness than at a later stage of the poisoning. One might then be inclined to speak of the initial increase and the following decrease in the responsiveness to the jarring. Perhaps some of the earlier investigators failed to consider this special phenomenon of the gradual decrease in irritability of the animals to the stimulus used and accordingly reported a heightened irritability and then depression of the poisoned animals when there was depression only. The fact that animals seem to become "accustomed" to the form of stimulus used has been called "forgetting with experience" by Piéron,³ and in an earlier work by one of us⁴ it was considered at length.

³ Piéron, H., *Archives de Psychologie*, No. 33, Tome IX, 1909.

⁴ Swindle, P. F., "Quantum Reactions and Associations," p. 45, 1922.

In order to offset, as best we could, the special difficulty encountered, the animal was stimulated five times per minute for two minutes, it was allowed to rest for fifteen minutes, the drug was applied to it, and it was then jarred at the usual rate for a period of two minutes. These time factors were varied considerably for the various animals. There was no instance in which the strychnin made an animal abnormally irritable. On the contrary, we were able to observe in many instances that a stimulus which was liminal for the normal animal was subliminal for the same animal shortly after the strychnin was applied. This was also the case generally when the later stages of poisoning were considered.

INCREASE IN NUMBER OF SPONTANEOUS CONTRACTIONS OF STALK IN FINAL STAGE OF POISONING

It was generally the case that immediately before death of the animals the cells underwent a characteristic vacuolation. Shortly after this vacuolation became perceptible the stalk began to contract more frequently. It was observed that animals which failed to contract in response to intense jarring as a stimulus contracted spontaneously at frequent intervals between stimulations.

In later experiments the animals were not jarred and only the spontaneous contractions of the stalks were counted. As these contractions of the stalk became more numerous they usually became smaller in amplitude, either because a portion of the stalk remained in a relaxed condition or because a part of it remained contracted in a coiled condition. In many instances, but not in all cases, the stalk was coiled and very short when the animal died, and it remained in this state of contraction. In view of the fact that this heightened activity occurs only after vacuolation commences and the animal is dying, it should be considered as the death struggle. Similarly, a skeletal muscle does some work of a special type while it is entering into the state of rigor. It may go into rigor either gradually or by contracting at intervals. It may lift and

sustain a considerable load, and during this time the skeletal muscle, like the vorticella, is practically non-irritable to external stimuli. Similarly, also, cardiac muscle may fibrillate shortly before it ceases action entirely.

It has already been mentioned that only a part of the stalk may contract and remain in the contracted or coiled state even long after the animal is dead. In many cases this contraction occurs in an unusual way, which was not observed while watching normal animals. The bell rotates slowly on its long axis and in the clockwise direction. As this occurs, a portion of the stalk coils and shortens. This passive response is therefore entirely different from the normal shortening of the stalk, and it should not be called an increase in activity, as has been done by some observers. In view of the facts that this passive "squatting" posture of the animal does not occur in response to jarring, that it occurs very slowly, that it is permanent and that it often occurs after the animal has ceased to respond in any other way, we must conclude that it is in no way comparable to strychnin spasms of the vertebrate. The stalks of some of the animals became entirely straight instead and remained in this condition long after the animals were dead, even long after the bell cytolized and only the stalk remained. It is significant that the elongated condition, as well as the described shortened one, can not be brought about by stimulating the animal; it shows that there is no connection between this behavior and typical strychnin spasms.

DEPRESSION OF THE CILIA OF THE VORTICELLA

In all cases the cilia appeared to be gradually depressed. In the final stages of the poisoning some of the cilia would cease to beat for a short time, while others continued their slow action; then the group which had stopped would begin and beat while others were still, and so on. This behavior, which was a result of depression and not of heightened activity of the cilia, was responsible

for somewhat periodic irregularities of movement of the bell. When we consider, however, that it was all due to such a state of depression of the cilia that some of them here and there on the bell ceased beating entirely from time to time and then beat feebly again after a shorter or longer pause, we are not justified in concluding that these irregularities in the behavior are in any way related to strychnin spasms of the vertebrate.

Two methods were employed in making the determination of depression of the cilia. One method consisted in observing the cilia directly. The cilia which vibrated so rapidly at first that their movements could not be followed with accuracy appeared to gradually slow down. Finally, their individual movements were easily observed. Of course it could not be determined definitely by this means whether or not there was an increase in the ciliary movements for a short time immediately after the drug was applied. The other method consisted in observing the rate of movement of diatoms and other small particles in the water in the neighborhood of the cilia. Some of these were driven in a circular or elliptical course by the cilia, and the number of rounds made in a given time by any one of them could be counted. Some of the bodies, however, moved rapidly toward the edge of the disc and continued to move past the edge in the same general direction, some of them in a parabola-like path until they were entirely outside of the water current created by the cilia. These bodies began moving more and more slowly soon after the strychnin was applied. When the cilia became depressed to such an extent that the individual movements could be observed there was very little movement of the foreign particles about the bell.

DEPRESSION OF OTHER UNICELLULAR FORMS

The vorticella was considered as being a very appropriate animal to observe in studying the effects of strychnin poisoning, because its stalk is irritable and contractile and because it is sedentary enough to be ob-

served well. Some of the non-stalked, free-swimming forms were comparatively poor as experimental animals because they were rarely quiet, for which reason it could not be determined with certainty that a change occurred in the activity in the initial stage of the poisoning. It was easily determined, however, that different varieties of the paramecium became much less active and ceased swimming some little time before they cytolized. It was evident that there was no final increase in the activity of the paramecium. There was likewise no final increase in the activity of the bell of the vorticella; the increase which was previously discussed was one of the stalk only. It was easily observed, especially in the case of some of the smaller ciliates, that the cilia at the different regions of the body were affected unequally by the strychnin. Some of the cilia were completely paralyzed, while some others were still feebly active, as was discussed at length by one of us in an earlier paper.⁵ This depression resulted in a certain asymmetry of movement of the animals.

One or two of the non-stalked, non-sedentary forms of the ciliate proved to be excellent experimental animals. One of these (*Oxytricha* sp. ?) resembles *Paramecium caudata* to a considerable extent, but the individual is much smaller and it swims and comes to rest frequently. It may lie quietly for a few seconds and then move rapidly to a new position, where it rests again, and so on. It might be said to jump from one place to another, but it really swims with its cilia. Regularly the number of jumps per minute were counted for ten successive minutes before the strychnin was applied, and after the drug was added the number of jumps per minute were again counted until the animal cytolized. The amount of movement at each jump was also considered. For every jump the animal made the observer made a vertical stroke on paper with a pencil. If the movement was exceptionally great a long stroke was made, a very small movement was

⁵ Kriz, R. A., This Journal, 1924, LVIII, 283.

indicated by drawing a very short line, and the intermediate amounts of movement were indicated on the data sheet by drawing lines of appropriate intermediate lengths. The results may be summarized as follows:

(1) The animals were poisoned and finally died when some small crystals of strychnin sulphate were placed carefully in the water, but there was no initial increase in activity.

(2) A general depression was the first effect of the strychnin. The animals were not only less irritable to external stimuli, but the spontaneous movements decreased in number.

(3) Between the time that vacuolation became easily perceptible and the time that cytolysis occurred, the jumps were unusually frequent. But these jumps were not normal ones. They were short and jerky, sometimes carrying the animal forward a very short distance, sometimes carrying it abruptly to the right or to the left, and at other times to the right or left over a spiral path. The nature of the responses was such that we believe the locomotor organs were depressed to such an extent that the normal coordination of the cilia was lacking. In fact, in the very latest stage of poisoning, groups of cilia at various regions of the body were observed to beat somewhat periodically and independently of the others. Some of the cilia acted alone and carried the animal a short distance in one direction, some others acted a little later and carried the body a short distance in another direction, and so on. This lack of coordination was due to depression and not to stimulation of the cilia.

(4) The poisoned animals were at all times less responsive than usual to such a mechanical stimulus as the jarring of the table. This condition was more definite in the later than in the earlier stages of poisoning.

SUMMARY

(1) There is at most only a superficial resemblance between the picture of strychnin poisoning of the unicellular forms studied and that of the vertebrate.

(2) The unicellular forms are rendered less responsive and the vertebrate first more and then less responsive to external stimuli.

(3) It was not possible to detect a state of heightened activity of the animals while they were reviving from the effects of strychnin after the drug was washed off in time for the animals to survive and approach their normal state of behavior.

(4) There was often an increase in the number of reactions of the animals during the processes of vacuolation and cytolysis. These are dying struggles, which are not comparable to the strychnin spasms of the vertebrate. In the first place, they are due to depression which occurs to such an extent that the movements are small and uncoordinated. In the second place, the animals are not more but less responsive than usual to external stimulation at this time.

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SHORTER ARTICLES AND DISCUSSION

THE VARIATION IN THE WIDTH OF THE ABDOMEN IN IMMATURE FIDDLER CRABS CONSIDERED IN RELATION TO ITS RELATIVE GROWTH-RATE

IN a recent number of the *AMERICAN NATURALIST*, Morgan (1923) discusses the variation in the abdomen-width of female fiddler-crabs (*Uca pugnax*?) with reference to the question whether they are so large as really to constitute variations in a definitive secondary sexual character, or whether they were not simply due to immaturity. His chief conclusions are as follows:

. . . at first the abdomen is narrow in both the male and the female. . . . The abdomen is still narrow in female crabs at a time when the first pair of claws in the male show that secondary sexual differences have already appeared. Later a certain stage is reached when the abdomen of the young females becomes much wider than that of the male; but not all the crabs of a given size in this period attain the full width of abdomen and in one species a few crabs may even become sexually mature with an abdomen less than full width.

I have recently been investigating this same problem from a somewhat different angle in the common British shore-crab *Carcinus maenas*. The results will appear shortly. The chief conclusions can be given very briefly, as follows:

(1) The most satisfactory method of expressing the result is to plot the ratio $\frac{\text{carapace width}}{\text{abdomen width}}$, as a percentage, against carapace width. This enables one to see at a glance whether any *relative* change in abdomen-width is occurring as growth progresses. If there is no such change, the graph is a vertical line. If this abdomen-width is increasing relatively faster than that of the carapace, the line will be inclined.

(2) Using this method, it is found that in ♂ *Carcinus*, the abdomen, after some early irregularities whose meaning is not yet clear, settles down to a rate of growth equal to that of the rest of the body (as measured by linear dimensions of the carapace). Females, on the other hand, although they are at the start externally indistinguishable from males, show from the outset of sexual differentiation (which of course occurs long before

sexual maturity) an increase of abdomen breadth which is greater than that of carapace breadth. Even in the largest female specimens so far found, the abdomen does not cover all the space between the bases of the legs. Thus, this relative increase of abdomen-width can, and actually does, continue *long after sexual maturity* is reached, as a normal occurrence, and in fact is going on throughout post-larval life.

(3) It is a matter of some interest that in the smallest crabs, from immediately after metamorphosis until the sexual differences in abdominal appendages appear (about 2 to 5 or 6 mm carapace length), the relative increase of abdomen-size with regard to body-size is already apparent.

(4) An inspection of the literature shows that there are numerous secondary sexual characters of Crustacea which behave in this way—*e.g.*, the chelae of many male decapoda described by Kemp (1913, 1914, 1915). These continue to grow relatively larger and larger the bigger the animal grows, long after sexual maturity has been reached. Geoffrey Smith some time ago (1905) drew attention to what is undoubtedly a corollary of this fact when he pointed out that in many forms—*e.g.*, the common stag-beetle (*Lucanus cervus*) the larger males possess the *relatively* larger “horns” (mandibles). With characteristic insight, he was not content to stop here, but went on to investigate the problem of the relative size of such secondary sexual characters in different-sized species and genera within a group. Here, again, he found that on the whole the larger-sized species had secondary sexual character which were not only absolutely but also relatively larger. This observation, which Smith made on Arthropoda, also applies to the Cervidae among the mammals, as a perusal of the literature has convinced me. (see especially the tables on ratio of antler-weight to body-weight in Rörig 1901.)

It appears, therefore (A) that when the secondary sexual characters of the two sexes of a species are distinguished by a quantitative difference, we shall find the difference brought about by a difference in relative growth-rate of the part in question over a considerable period of development—*i.e.*, in one sex that part will grow more in unit time than does the rest of the body, or the same part in the other sex.

(B) This difference in relative growth-rate may continue throughout life, as in the abdomen of ♀ *Carcinus* or the chelae of many ♂ prawns. Or it may cease at or near sexual maturity,

as in the abdomen of ♀ *Uca*, although general growth is continued. Or it may cease because general growth ceases, as in higher vertebrates or in insects.

(C) The relative size of some secondary sexual characters which appear suddenly, like those of insects in the imago form, may be determined by the relative extent to which certain chemical processes have proceeded during previous development; and when these processes take place relatively faster than those leading to the growth of other parts of the body, we again find the secondary sexual characters relatively larger in large specimens (Stag-beetles, etc.).

(D) Because this mode of development is apparently the primitive and simple one for various characters, it follows that wherever characters are found which depend for their formation upon an increased relative growth-rate, whether throughout life or for all or most of the immature period, then we shall find such characters on the whole relatively more developed in larger than in smaller species within the same group; *i.e.*, the fact that such characters are relatively larger in the larger species is a case of orthogenetic evolution—the character “greater relative size of sexual characters” is not determined primarily by selection, or by Lamarekian means, but by the prior existence of a determinate type of growth-mechanism in the organization of the group.

I have applied this method of calculating the relative growth-rate of the abdomen to the data presented by Morgan in his correlation table on p. 276. I at first calculated the ratio $\frac{\text{abdomen width}}{\text{carapace width}}$, expressed as a percentage, for each of the classes of carapace-width given by him.

When these results were plotted (graph not here figured), it was seen that some points were very aberrant, especially those derived from the classes with few individuals. Accordingly, the figures were reclassified into 5 classes: (1) 4–6 mm, (2) 6.5–8.5 mm, (3) 9–10 mm, (4) 10.5–11.5 mm, and (5) 12–14 mm carapace-width, all inclusive. The mean carapace-width and mean, maximum and minimum $\frac{\text{carapace width}}{\text{abdomen width}}$ ratio were then calculated for these classes.

The result is given in Tables I and II.

When these figures were plotted, the graphs were very regular. They are reproduced in Fig. 1. The chief points that emerge are

TABLE I

Carapace width mm	Number of specimens	100 $\left(\frac{\text{Abd:width}}{\text{Car:width}} \right)$		
		(a) mean	(b) maximum	(c) minimum
4	2	37	37	37
4.5	2	28	33	22
5	9	36	40	30
5.5	2	36	36	36
6	5	35	42	33
6.5	5	38	46	31
7	10	40	43	36
7.5	12	40	47	33
8	14	41	50	38
8.5	13	47	65	35
9	27	54	67	44
9.5	19	59	63	42
10	52	63	75	45
10.5	36	64	71	48
11	46	65	73	50
11.5	34	67	74	57
12	57	67	75	58
12.5	21	66	72	56
13	23	67	69	58
13.5	6	67	67	67
14	7	64	64	61

The maximum and minimum figures are those of the single individual with largest and smallest relative abdomen-breadth in each class.

TABLE II

Carapace width		Number of specimens	100 $\times \frac{\text{Abdomen width}}{\text{Carapace width}}$		
(a) inclusive	(b) mean		(a) mean	(b) maximum	(c) minimum
4 - 6	5.1	20	36	42	22
6.5- 8.5	7.7	54	42	65	31
9 -10	9.6	98	59	75	42
10.5-11.5	11.0	116	65	74	48
12 -14	12.4	114	67	75	56

as follows. In the first place, the general slope of the curve for the mean ratio shows that relative increase of abdomen-width is continued throughout the whole of the period to which the measurements refer—*i.e.*, from a carapace width of 5 to one of about 12.5 mm. This is indicated in Morgan's correlation table (his Table I) by the shape of the column of figures, which presents a slight concavity downwards and to the left. But this is so small that it would be difficult to draw any conclusion from it. The method of plotting the percentage ratio at once reveals what is happening, and also shows that the relative rate of growth of the abdomen appears not to be constant; it is slower at first, then increases to a maximum in crabs of 8 to 10 mm carapace breadth, and then decreases again, becoming at the end

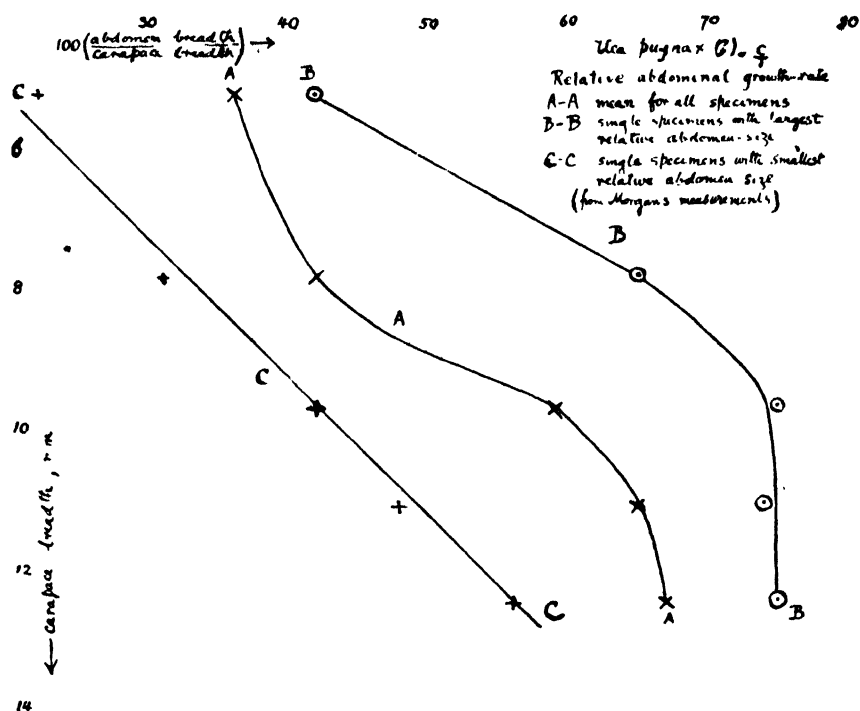


FIG. 1

almost the same as the rate of increase of the carapace. (See below for further discussion).

So far we have been considering the average. The curves for the maximum and minimum abdominal ratios give us the limiting values and throw further light on the problem. (1) The slope of the "maximum" curve during the period of greatest relative abdomen-growth is the most nearly horizontal of the three curves—i.e., "maximum-abdomen" crabs are those with the quickest relative abdomen-growth. (2) However, this means that the abdomen reaches full relative size earlier; therefore, the "maximum" curve bends sharply over before 10 mm carapace breadth, and then continues vertically downwards; i.e., in crabs with rapidly-growing abdomens, a stage is reached well before full body-size after which the abdomen does not grow faster than the rest of the body, but at the same rate as it. (3) The minimum curve, on the other hand, continues to show the same slope throughout, i.e., it comprises such crabs as have such a slow relative increase of abdomen-size that full abdomen-size is not reached by animals of the sizes obtained by Morgan.

We may sum all this up by saying: (1) That the broad abdomen of female fiddler-crabs develops gradually from a condition in which it is no broader than that of the male, by showing for a long period of time a growth-rate higher than that of the body as a whole. Pézard (1918) calls such a growth-rate *heterogonic*, and the term is one which will be found useful. (2) That this abnormally high growth-rate comes to an end when the abdomen covers all the ventral surface of the thorax between the bases of the legs. Such abdomens Morgan calls "full width;" this of course means full relative width, not full absolute width. After this point the abdomen grows at the same rate as the rest of the body. (3) That the relative abdominal growth-rate varies considerably in different individuals.

It is this last fact which leads to the finding of different degrees of abdominal development in crabs of one size. However, since the rate of abdominal growth continues to be considerably greater than that of the rest of the body up to the largest crabs measured, in precisely those specimens which possess the smallest relative abdomens, it seems legitimate to suppose that "full width" abdomens are the normal end-result of development in *all* individuals. Of course, if the relative abdominal growth-rate is low enough, death by accident or old age may normally supervene before "full width" is attained. This, apparently, is what occurs in *Carinus macnas*. It would be interesting to collect the largest female *Uca pugnax* discoverable and see whether all finally attained in the normal course of things to "full width." It is not clear from Morgan's statements at what size sexual maturity normally begins, and what is the maximum size attained. These points should also be determined.

One detail demands consideration—the S-shape of the curve for the mean ratios. From the shape of the "maximum" and "minimum" curves, one would expect that the "mean" curve would, until it began to bend over toward the vertical, be a straight line of slope intermediate between the other two. The kink observed may be (1) an expression of a real change in relative growth-rate at that particular size or (2) at a particular season, or (3) it may have something to do with the fact that the crabs, as Morgan states on his p. 275, were collected from two quite remote localities. Further measurements based on uniform material and on considerably larger numbers for the smaller-sized crabs would probably solve the problem.

Morgan (1923, p. 274) quotes the general conclusion reached in his previous paper (1920). He says, "These crabs [the ♀ ♀ with abnormally small abdomen] showed an apparent change towards maleness, or possibly a retention of the juvenile condition." On p. 275, he sums up his later paper by saying that "they therefore represent, as I supposed, a variation in a secondary sexual character"—meaning by this, I take it, what he previously referred to as a variation of the female in the direction of the male type.

I think it will be clear that the method of analysis adopted in the present paper shows that this involves a much too static way of looking at the problem. I take it that the abdomen of male fiddler crabs, like that of *Carcinus*, will be found to have the same rate of growth as the rest of the body. Even the females with minimum relative size of abdomen, however, show the chief female characteristic in respect of this character, *viz.*, a growth-rate of abdomen greater than that of the rest of the body; the relative abdominal growth-rate is merely slower than in other females. Thus Morgan's statement (p. 283) that "there is much variation in the extent of the change" (from narrow to broad abdomen in ♀ ♀, merely needs rewriting with *rate* substituted for *extent*).

This implies, it is true, a slight quantitative variation in the direction of the male, but *qualitatively* all such animals remain typically female. They display the typical ♀ mode of abdominal development, of which the normal male shows no trace—the one is heterogonic, the other isogonic.

In a similar way, Pézard (1918) has shown that the comb of normal ♂ fowls is heterogonic—grows at a much greater rate than the rest of the body; while that of capons grows at the same rate. Or again, the limbs of thyroidectomized tadpoles grow certainly no faster than the rest of the body, whereas those of normal tadpoles grow faster. Here, further, we are introduced to more detail, for within certain limits greater concentration of thyroid hormone in the body causes more rapid relative growth of limbs; and also the considerable differences in length of time (under comparable conditions) taken by different individuals of the same species, and by different species of the group, to reach the time of metamorphosis undoubtedly depend on variations in the relative growth-rate of the thyroid—more accurately, on the rapidity of the processes leading to the production of thyroxin

considered in relation to the processes of general body-growth of the tadpole. Such variations in the time of metamorphosis among the individuals of a species are strictly comparable from the point of view of developmental physiology with the variations observed by Morgan in the breadth of his crabs' abdomens, since both depend upon variations in the relative rate of two connected processes of development.

SUMMARY

By re-plotting Morgan's data on the growth of the abdomen in female fiddler crabs, it is shown that the differences in abdominal development which he regards as implying variations toward the male type, in reality represent only a slower relative abdominal growth-rate. The abdominal growth-rate of females is always *heterogonic* (Pézard) while that of males, if we may judge from other species, is *isogonic*.

The best method of detecting and analyzing heterogonic growth-rate is by plotting the percentage size of the part in question against the absolute size of some dimension of the whole body.

The bearing of the existence of heterogonic growth-rate upon certain general problems is briefly touched upon.

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NEW COLLEGE, OXFORD,
AUGUST, 1923.

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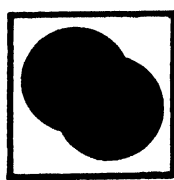
TRIHYBRID BLOCKS

A PEDAGOGICAL device for visualizing the possible combinations in the F_2 generation of a trihybrid may be made with sixty-four similar cubical wooden blocks, preferably one inch in each dimension.

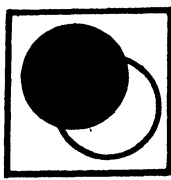
Three arbitrary symbols, squares, circles and triangles, that may represent any actual characters as desired, are painted on the different faces of the cubes, the opposite faces in every instance being alike.

On each face the symbols appear double, one partially overlapping the other, to represent the zygotes formed from two parental gametes. When the two symbols on any face are filled in solid black they represent the duplex or dominant homozygote. The corresponding nulliplex, or homozygous recessive, is represented by the overlapping symbols drawn in outline, while the simplex (hybrid) heterozygote is shown by the symbol in outline overlapped by the symbol filled in solid.

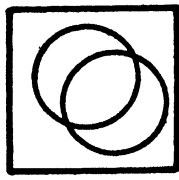
For example, in the case of circles the symbols would appear as below:



Duplex



Simplex



Nulliplex

How to make each face of each of the sixty-four trihybrid cubes can be determined from the appended checkerboard of a theoretical trihybrid. In this the symbols to be painted on the blocks correspond to the letters as follows:

C = solid circle.

c = outline circle.

T = solid triangle.

t = outline triangle.

S = solid square.

s = outline square.

Since opposite faces of each cube are alike, any angle at which a block is viewed so as to show three faces will read like the three faces seen from any other possible angle. In the checkerboard the upper left hand block represents a triple homozygous dominant and the lower right hand a triple homozygous recessive. If two individuals represented by these symbols are crossed the offspring, as we know, will be heterozygous or hybrid for each of the three characters in question. When two such trihybrids are

	CTS	CtS	CTs	Cts	cTS	ctS	cTs	cts
CTS	CC TT SS	CC Tt SS	CC TT Ss	CC Tt Ss	Cc TT SS	Cc Tt SS	Cc TT Ss	Cc Tt Ss
CtS	CC tT SS	CC tt SS	CC tT Ss	CC tt Ss	Cc tT SS	Cc tt SS	Cc tT Ss	Cc tt Ss
CTs	CC TT sS	CC Tt sS	CC TT ss	CC Tt ss	Cc TT sS	Cc Tt sS	Cc TT ss	Cc Tt ss
Cts	CC tT sS	CC tt sS	CC tT ss	CC tt ss	Cc tT sS	Cc tt sS	Cc tT ss	Cc tt ss
cTS	cC TT SS	cC Tt SS	cC TT Ss	cC Tt Ss	cc TT SS	cc Tt SS	cc TT Ss	cc Tt Ss
ctS	cC tT SS	cC tt SS	cC tT Ss	cC tt Ss	cc tT SS	cc tt SS	cc tT Ss	cc tt sS
cTs	cC TT sS	cC Tt sS	cC TT ss	cC Tt ss	cc TT sS	cc Tt sS	cc TT ss	cc Tt ss
cts	cC tT sS	cC tt sS	cC tT ss	cC tt ss	cc tT sS	cc tt sS	cc tT ss	cc tt ss

crossed, making the F_2 generation, the sixty-four possible combinations resulting are shown by the sixty-four cubes.

Various other things may be illustrated by these blocks. For instance, they may be stacked so as to show that a trihybrid is simply three monohybrids combined.

To do this first spread the blocks on a table with one set of symbols face up—for example, the squares. Then assort them into four groups of sixteen each representing the F_2 generation of a monohybrid. One group will be entirely made up of double black squares (dominant homozygotes); two groups will each show one black square overlapping an outline square (dominant heterozygotes) and the fourth group will be double outline squares (recessive homozygotes).

Regarding the groups as units the typical Mendelian proportion of 1:2:1 for a monohybrid is thus graphically demonstrated. Each of these four groups of sixteen may now be arranged in turn, leaving the squares up, in four rows of four each so that they likewise read by rows 1: 2:1, in one direction for circles and in the other for triangles. The four groups of six-

teen each may next be superimposed to form a cube containing all the sixty-four blocks, reading independently in any plane as Mendelian monohybrids. Likewise any four blocks that are now side by side when taken out from the cube of sixty-four will read 1: 2: 1 like a monohybrid. Thus the fact that a trihybrid is made up of three independent monohybrids may be demonstrated.

It will be found that the eight blocks in the center of the stack are all alike, being hybrid for each of the three characters used.

Second, the Mendelian explanation of apparent blending inheritance by means of duplicate genes may also be shown by rearranging the blocks. To do this each solid filled-in symbol, regardless of its shape, should be regarded as a duplicate gene of the character in question. The blocks may now be lined up according to the number of duplicate genes which each presents, that is, the number of solid symbols showing on any three continuous faces (the opposite faces being alike).

There are seven possible lines in this classification from 0 to six inclusive and these will be found to form a regular variability curve as follows:

Number of blocks	1	6	15	20	15	6	1
Number of duplicate genes	0	1	2	3	4	5	6

These sixty-four blocks represent the F_2 offspring from two unlike grandparents, one with six duplicate genes of the character in question and one with 0 genes. The F_1 hybrids have three genes or a blend $\left(\frac{0+6}{2}=3\right)$ and twenty out of sixty-four of the F_2 generation are likewise intermediate blends with three duplicate genes like their parents, while thirty others are nearly like the parents, having either two or four duplicate genes instead of three.

This large number of F_2 offspring blending the grandparental characters like the parents has given rise to the impression that all the F_2 generation blends.

The arrangement of the blocks, however, as well as a careful analysis of actual results of experimental breeding reveal the variability curve in the F_2 generation which indicates that supposed cases of blending inheritance may receive a satisfactory explanation upon a strictly Mendelian basis.

H. E. WALTER

OPISTHOTONIC DEATH IN A SALAMANDER¹

SOME time ago attention was directed by R. L. Moodie² to the fact that in fossil vertebrates the skeletal remains are sometimes so disposed as to suggest the possibility of death due to or occurring in connection with diseased states resulting in opisthotonic distortion. Dean objected³ to such interpretation, on the ground that among lower vertebrates opisthotonic death has seldom (if ever) been recorded. An "accidental experiment" involving a diseased salamander has given me a chance result which seems worth noting in connection with this interesting discussion.

About 60 salamanders, obtained a few hours before, were placed by the collector in a small aquarium with about two inches of water, and through accident were not properly tended, so that on the next morning they were found dead by suffocation in the foul-smelling medium which by that time had resulted. The animals were for the most part *Plethodon glutinosus* and *P. erythronotus*, with a few *Spelerpes ruber*. One *P. glutinosus* had been noted in the field as showing an extensive loss of skin over the anterior portion of the body. The appearance was such as to suggest the effect of a skin disease. This individual had been quite sluggish and by comparison with its fellows was regarded as "sick." This individual was among those found dead. Its condition made it strikingly conspicuous among the several dozen others, for it was in pronounced opisthotonic curvature. This attitude was in no degree suggested by the posture of any of its companions, which were quite straight and with the limbs at random angles. The diseased animal, together with a number of the others, was preserved in alcohol.

Photographs of the opisthotonic individual are shown in Figure 1. The area denuded of skin is clearly visible. The dorsal curvature of the head and tail is marked, as also the rigid extension of the limbs and digits. For comparison views are also given (Figure 1) of the most nearly "opisthotonic" of the numerous now-diseased individuals. All were in rigor, resting on the bottom of the aquarium, when preserved.

¹ From the Zoological Laboratory, Rutgers University.

² AMERICAN NATURALIST, Vol. 52, pp. 384-394, 1918; *Science*, N. S., Vol. 50, pp. 275, 276, 1919. See also "Paleopathology," Univ. Ill. Press, 1923, p. 567 [Chapt. X].

³ *Science*, N. S., Vol. 49, p. 357, 1919.

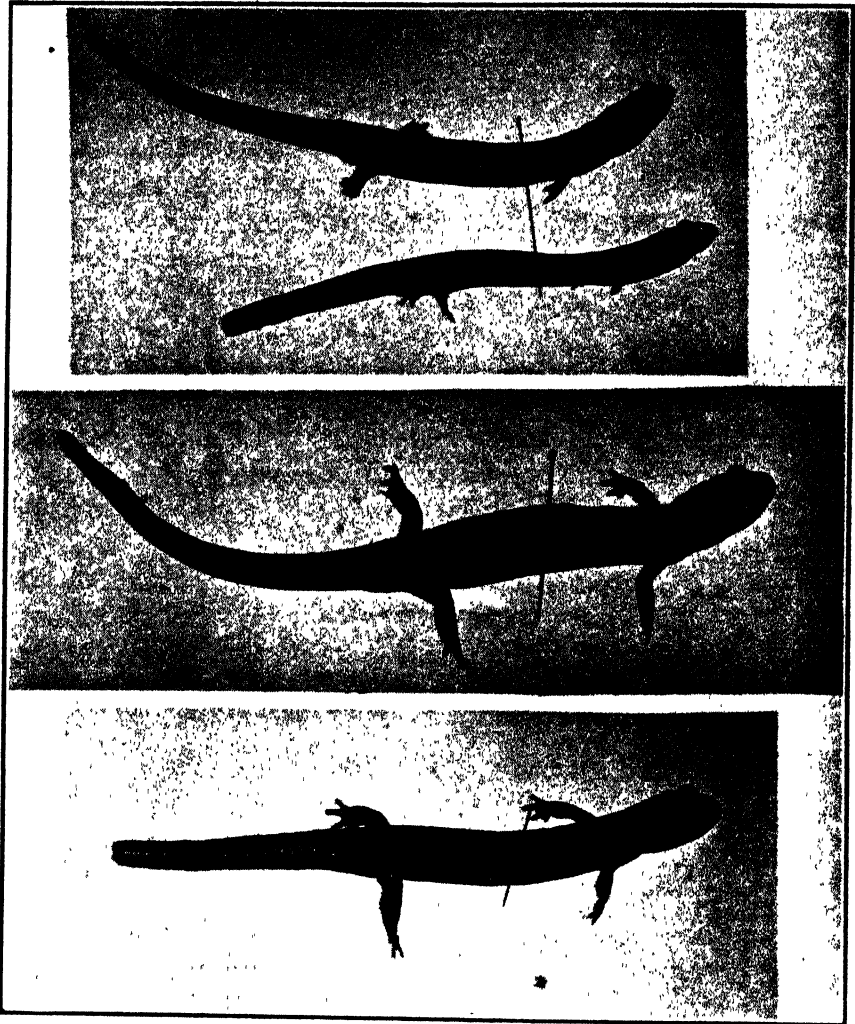


FIG. 1. Above, lateral views of opisthotonic diseased salamander with eroded skin area, and of a normal specimen dead under same conditions. Below, dorso-lateral views of the same, at greater enlargement. Photographs by Dr. L. A. Hausman.

It is not unreasonable to regard this finding of opisthotonic distortion in a diseased salamander, under fatal conditions which failed to elicit such phenomena in a good number of its normal companions (of the same and of other species) as perhaps significant for the question which Moodie has raised.² More recently I have observed pronounced opisthotonic posture in a few (but not in all) individuals of the horned toad *Phrynosoma cornutum* which had died in a terrarium.

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THE STABILITY OF SUBSPECIFIC CHARACTERS UNDER CHANGED CONDITIONS OF ENVIRONMENT

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INTRODUCTION

IN various recent papers I have discussed the geographic variation of mammals and birds, and particularly the marked correlation which exists between certain characters of local races and features of their physical environment. While this last fact has long been recognized by naturalists, and has been the subject of considerable theoretical discussion, the problems here raised have rarely been subjected to experimental test.

Not many years before the present studies were commenced, it was possible for Jordan to write:

It remains, however, to be determined whether these environmental forms—these species and subspecies produced by the direct influence of heat, cold, humidity and aridity—are “ontogenetic species” . . . or whether they have a real existence outside the lifetime of the individuals actually composing the group or species. We do not know which of the traits induced by direct action of the environment, if any, are actually hereditary and which are not.

Referring to certain possible experiments with woodpeckers, Jordan argues that if the parental color tone were retained in the progeny of a stock which was transferred from Vancouver to Arizona, “then humidity would be a real factor in the formation of species.” If, on the other hand, the transplanted birds “should develop in the fashion of the local race of this region . . . then the duskiness is not a true specific or subspecific character. . . . It may be that these questions have been

already solved by experiments on birds, but if so, the experiments have escaped my attention."

The writer thereupon suggests some profitable undertakings with birds, remarking in this connection: "Perhaps our ornithologists will some day test their species and subspecies by a test of the permanence of this class of characters.¹

In a reply to this significant utterance by Jordan, J. A. Allen lays stress upon the fact "that the local differentiation in color between the subspecific forms of a given group is often (but not always) much more strongly expressed in the first pelage or plumage of the young than in the adults of the same forms." This he regards as sufficient evidence "that local differentiations are transmitted from parent to young, and are hereditary in the usual sense of that term." He continues:

Doubtless no one questions their continued transmission from generation to generation so long as the environment remains stable. Probably also few would question that were representatives of a strongly marked local form . . . to be transplanted to a region markedly different climatically from their natural home, they would gradually lose their original characteristics and become, after a number of generations, more or less modified, in better agreement with the new conditions of life. But it would be apparently rash to expect a very material change in a single generation.²

Allen is, however, unable to offer any exact positive evidence on the subject unless we except the single case of a quail which had been introduced (presumably) from Florida into Cuba, and which, after a hundred years, was said still to be "impossible to distinguish from the Florida bird," thus differing "in a marked degree" from the Cuban form.

These statements by Jordan and Allen bear testimony to the lack of critical evidence in this field as late as 1906.³ Even at the present day, indeed, doubts are occasionally expressed as to the fixity of subspecific characters, particularly among birds. Thus only three years

¹ *Science*, December 29, 1905.

² *Science*, January 26, 1906.

³ Vaughan (*Science*, May 4, 1906), in this same year, asks some pertinent questions of a similar nature to Jordan's.

ago, Lowe and Mackworth-Praed⁴ expressed themselves as follows:

We think there can be little doubt that many—indeed, by far the majority—of our present-day subspecific forms belong to this last category [“fluctuational” and non-heritable, as contrasted with “mutational”] and are mere environmental, unstable and essentially superficial variations, which would quickly disappear if the organism were transferred from its normal environment to some other of a different nature.

Of the various interrelated questions which demanded solution, the first and most obvious, of course, was whether these subspecific differences were inherited at all. Would they persist *to any extent* if two different geographic races were reared in a common environment, or might not all subspecies prove to be purely “ontogenetic,” in the sense in which Jordan (following Kellogg) employed that term?

At the close of 1913, opportunity was offered the present writer by the Scripps Institution for Biological Research to put this question to an experimental test. The most abundant, variable and widely distributed species of deer-mouse, *Peromyscus maniculatus*, was chosen as the most promising material for this investigation. In the spring of 1914, I trapped a preliminary series of living *P. m. sonoriensis*, in the Mojave Desert, near Victorville, California, and took these to Berkeley for breeding purposes. In August, 1915, I was able to report that “*neither the originally introduced animals nor their offspring, nor their grandchildren, have thus far shown any perceptible approach to the local type [gambeli].*”⁵

In 1915, the *Peromyscus* studies were transferred to La Jolla. The material employed in the transplantation experiment now included specimens of a dark race, *P. m. rubidus*, from the redwood forests of Humboldt County, California, as well as a fresh series of *sonoriensis* from Victorville. In 1917, it was possible to state, for both of these subspecies, that no evidence of color change had

⁴ *Ibid.*, April, 1921 (this article was prompted by one by L. M. Loomis, expressing a similar viewpoint).

⁵ AMERICAN NATURALIST, November, 1915.

been detected, although they had been bred as far as the third cage-born generation.⁶

This was not true, however, of some other characters. It early became evident that cage-bred mice, belonging to all these geographic races, tended to be stunted more or less by some unknown influence pertaining to the conditions of captivity. This stunting affected the relative length of certain appendages, as well as the size of the body as a whole, and thus led to the modification of some of the very characters which are of most importance in the comparison of subspecies. It was evident, however, that the local race, *gambeli*, was modified in the same direction as the two introduced races, *rubidus* and *sonoriensis*. The measurement of considerable series, belonging to each of these subspecies, showed that the relative positions of these three races were but slightly affected by the changes in question. There was found, it is true, some degree of convergence on the part of *rubidus* toward *gambeli*. But the fact that *sonoriensis* was found to *diverge more widely* from *gambeli* in the C₂⁷ generation than in the parent generation deprived the former fact of all significance.⁸ That there is, in general, no tendency toward a convergence of the introduced types will be shown later.

The results of these simple experiments made it plain that for one species of *Peromyscus*, at least, subspecific differences are to a large extent hereditary. Without going further, the evidence was sufficient to refute the supposition that these differences are wholly, or even chiefly, the result of climatic or other conditions, acting within the lifetime of the individual.

There remained, however, the important question of the degree of stability of these subspecific characters.

⁶ Bulletin of the Scripps Institution, No. 3, October 19, 1917 (University of California Press).

⁷ The various cage-born generations have been designated C₁, C₂, etc.

⁸ AMERICAN NATURALIST, June-July, 1918, p. 292. The pathological nature of the changes in this *rubidus* stock was likewise shown by the fact that the strain became so largely sterile that it was impossible to continue it further. The behavior of a normal strain will be discussed below.

The data thus far cited are plainly not adequate for the answer of this question. A slight tendency toward convergence on the part of the introduced races might well fail of detection in the absence of sufficient numbers of specimens. Or the effects of the local climatic conditions might be masked by the deformations due to captivity. For the purposes at hand, it is evident that we need larger series of more normal animals, a greater number of generations and more precise quantitative treatment of the material. In the case of the data next to be presented, these conditions have, to a considerable extent, been realized.

LATER TRANSPLANTATION EXPERIMENTS

Before considering the more precise data from these later experiments, it will be well to discuss briefly some of the climatic differences between the localities here considered. My thanks are due Professor H. H. Collins, now of the University of Pittsburgh, for important assistance in computing the meteorological data here offered.

Victorville is situated near the western border of the Mojave Desert, at an altitude of 2,700 feet, and about twenty miles from the mountain barrier which divides the desert from the coastal plain. La Jolla is situated directly on the ocean shore, about fifteen miles north of San Diego. Carlotta lies near the opposite end of the state, in a district until recently occupied everywhere by dense redwood forests. The region is one of fairly high rainfall, frequent heavy fogs and overcast skies.

In Table I are summarized certain meteorological data for these three regions.⁹ There are several salient points revealed by a comparison of these figures: (1) In respect both to atmospheric humidity and to rainfall, the desert station represents the extreme of aridity, and that of the northwest coast the extreme of humidity, with La Jolla occupying an intermediate position. It is probably no mere coincidence that in respect to depth of pigmenta-

⁹ Data for Eureka, rather than Carlotta, are given, owing to lack of records for the latter point. The two are about twenty miles apart.

tion, the three local races of *Peromyscus* follow the same order, the desert race being the palest. (2) In respect both to daily and seasonal range, and to that of temperature as well as that of humidity, the figures for the desert are far in excess of those for the northwest coast, La Jolla again being intermediate. (3) In respect to mean annual temperatures, the order is somewhat different, La Jolla and Eureka representing the extremes, while Victorville is intermediate. Although deserts in general are regions with high summer temperatures, this fact is counterbalanced by their relatively low winter temperatures. That the mean temperature of Victorville is actually lower than that of La Jolla is not surprising in view of its greater elevation and higher latitude.

TABLE I

CLIMATOLOGICAL DATA FOR LOCALITIES CONSIDERED IN THE PRESENT PAPER¹⁰

	Temperature			Relative Humidity			Rainfall
	Annual mean (° C.)	Mean daily range	Seasonal range (difference between highest and lowest monthly mean)	Annual mean	Mean daily range	Seasonal range (difference between highest and lowest monthly mean)	Annual mean
Victorville...	14.0	20.4	20.4	49.8	57.0	14.5	6.2
La Jolla	16.3	7.4	10.4	73.8	24.1	11.1	10.0
Eureka . . .	9.8	6.1	8.1	86.7	15.8	9.4	46.0

¹⁰ Temperature and humidity data are based upon records of thermographs and hygrographs, checked by mercury thermometers. Records were kept at Victorville, from December, 1914, to January, 1917, inclusive, the instruments being in charge of Mr. Ralph H. Webb. At Eureka, the records were kept from November, 1914, to January, 1917, inclusive, Mr. B. S. Nichols, of the U. S. Weather Bureau, having charge of the instruments. At both of these stations the instrument shelters were purposely placed much nearer the ground than is done when "standard" meteorological observations are undertaken. At Eureka, the instruments were placed among underbrush, in a strip of redwood forest.

At La Jolla, meteorological instruments were installed in the murarium, almost from the commencement of these experiments. Owing to the construction of this building the temperature and humidity conditions are very

Peromyscus maniculatus sonoriensis: The parent stock of this series was trapped near Victorville, in April, 1915, and was brought at once to La Jolla. For six successive generations these mice were reared in the "murarium,"¹¹ in our standard laboratory cages, having the dimensions 16 x 9 $\frac{3}{4}$ x 9 $\frac{3}{4}$ inches. The stunting effects of captivity were manifest, to some extent, even in the C₁ generation, while measurements of the C₂ generation showed that the mice had undergone a considerable reduction in mean body length, as well as in the mean size (both relative and absolute) of some of the measured parts.¹²

Fifteen animals (3 ♂, 12 ♀), belonging to the C₅ generation, and 13 (3 ♂, 10 ♀), belonging to the C₆ generation, were transferred to two of our open pens, August 18, 1919. The pens in question each have an area of about 12 $\frac{1}{2}$ x 12 $\frac{1}{2}$ feet. They are covered by two layers of wire screen, and are bounded, beneath the ground, by concrete walls, sunk to such a depth as to prevent burrowing animals from entering or leaving them. The floor of these pens is constituted by natural soil, into which

nearly the same as in a "shelter" of the usual pattern. Records for three years (September, 1915, to August, 1918) were utilized in obtaining the present humidity data, while only two of these years were used in the case of temperature.

Annual means, both for temperature and humidity, were obtained by averaging the daily means, these last, in each case, being the mean of the daily maximum and minimum. The laborious task of digesting these records and making the necessary computations was performed by Dr. H. H. Collins.

The recording apparatus, though adjusted at times, and checked at weekly intervals by more reliable instruments, was subject to variable and frequently considerable errors. Comparison with Weather Bureau records for Eureka and San Diego reveals, however, a rather unexpected degree of correspondence between our records and the official ones. While doubtless not adequate for exact meteorological studies, the data here given certainly suffice to show the climatic differences between the localities in question.

The figure for rainfall at Victorville is based upon rain-gauge records kept for 11 years by Mr. Reginald Frost. For La Jolla, the figure for San Diego has been employed. The Eureka figure is based upon Weather Bureau records for that station.

¹¹ This building is so constructed that the outside atmosphere circulates freely through it.

¹² AMERICAN NATURALIST, June-July, 1918, pp. 290-293.

the mice burrow freely. Food is brought them twice weekly.¹³

At the time of this transfer, the C₅ animals were 10 to 11 months old, the C₆ ones being, for the most part, three to four months old. There was considerable stunting and some actual deformity (*e.g.*, curved feet) among the *sonoriensis* stock at this time. It is probable that the more normal individuals were selected for the pens.

It should be superfluous to state that pedigree breeding, such as is practiced habitually in our small cages, is impossible in these large inclosures. Ordinarily, it is not even possible to know the generation to which a given individual belongs.

The *sonoriensis* stock was kept in the pens referred to for a period of nearly four years, in the course of which period the animals were all trapped out twice and part of the stock eliminated. On each of these occasions, the lot which was returned comprised none of the individuals which had been introduced on the previous occasion.¹⁴ Since the original lot of mice which was placed in the pens belonged to the C₅ and C₆ generations, it is evident that the lowest possible number of generations represented by any mouse at the end of the experiment was seven, with a probable minimum of eight generations for half of the stock. It is quite unlikely, however, that these minimum figures indicate fairly the average number of generations which were produced during the period of the experiment. They leave out of account the fact that many of the mice were known to be breeding actively during this period, and that *Peromyscus* sometimes begins to reproduce at the age of 3 or 4 months.

It would be a conservative statement that the specimens of *sonoriensis* which were measured and skinned in October, 1923, represent a minimum of seven generations bred at La Jolla, and a maximum of twelve or more, while the majority probably belonged to the eighth to the tenth generations.

¹³ They are not fed daily owing to the distance of these pens from the other buildings of the station.

¹⁴ Recognition of these was possible by means of identification marks.

Fifty-three specimens (18 ♂, 35 ♀) were chosen for measurement. Save that obviously immature individuals and those with damaged pelages were rejected, care was taken that the choice of specimens should be random.

The occupants of these pens are much prone to fighting and inflict many minor injuries upon one another. In particular, the tail and rump regions suffer most from this persecution. Specimens with tails more or less ab-such tails can not, of course, be included in our computations. For this reason, it has been necessary to reject the tail length of 5 *sonoriensis* and 19 *rubidus*.

Injuries to the skin frequently leave lasting effects, even when healing is complete. Thus damaged areas may show pale spots, due to the replacement of normal, colored hairs by white ones. Careful examination reveals the presence of such spotting in a considerable proportion of pelts from the open yard stock, the posterior dorsal region of the skin being most affected. When these traumatic changes are sufficiently pronounced, they appreciably affect the coat-color, and it has been necessary to reject such specimens from our series. The *sonoriensis* stock has suffered much less in this respect than the *rubidus* stock.

For the purposes of the color analysis, a rectangular area of standard size (31 x 17 millimeters) and nearly constant position was subjected to examination in each case. The rectangle chosen was situated transversely, across the posterior dorsal region of the pelage, and a little in advance of the base of the tail. The color determinations were made with the Hess-Ives tint photometer, the use of which instrument for the study of mammalian coat-color has already been discussed in several previous papers.¹⁵

In Table II are given the means and standard deviations for the four values which have been considered

¹⁵ *Journal of Mammalogy*, May, 1921 (the procedure there given has been somewhat modified); *Journal of Experimental Zoology*, October, 1922; *ibid.*, October, 1923.

throughout these studies. The first line of figures are those for a series of wild specimens of *sonoriensis* which had been trapped at Victorville.¹⁶ This lot includes some of the actual ancestors of the experimental series. In the second line are the figures for this same race, after a residence of more than 8 years at La Jolla, and the lapse of from 7 to (probably) 12 or more generations. All the latter animals and a large majority of the former were killed in the fall (September and October).¹⁷

TABLE II

COAT-COLOR ANALYSIS OF *PEROMYSCUS MANICULATUS SONORIENSIS*

	No.	Black		White		Color		Red : Green	
		Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Wild ¹⁸ ..	49	77.14 ± .21	2.22	11.94 ± .11	1.19	10.91 ± .15	1.53	2.87 ± .02	.22
Trans-planted	47	75.69 ± .18	1.86	13.66 ± .12	1.25	10.65 ± .14	1.47	2.81 ± .02	.22

It will be seen that there are small though significant differences between these two series in respect to the proportions of black and white. This corresponds to the

¹⁶ In order to be sure of the full maturity of these specimens, only those were included which had been kept for 4 months or more after capture.

¹⁷ Somewhat more favorable skins would have been obtained had all the animals been killed after the completion of the fall molt. Many skins belonging to both series give evidence of molting on the reverse side, though the color tone of the pelage is seldom materially affected thereby.

Winter pelages appear to average a trifle darker, in this species, than those of the summer and fall, a fact which may account for a small part of the difference between the two series comprised in Table II. The relative values are but slightly changed, however, when the comparison is restricted to fall pelages.

¹⁸ These figures for the "wild" mice differ somewhat from those published in a previous paper (*Journ. Exp. Zool.*, October, 1923) for the same series. This is due to the fact that they represent a later set of readings, made at the same time as those for the "transplanted" series. Owing to the influence of atmospheric conditions upon photometer readings of these skins, strictly comparable figures can not be obtained unless the conditions are identical. It is my practice, when two series are to be compared, to repeat each of the readings on a different day, so alternating the two lots as to distribute equally the effects of any such disturbing factors.

fact that the "transplanted" series, even to the unaided eye, averages somewhat paler than the parent ("wild") lot. The proportions of "free" color are, however, closely alike in the two series, while the spectral position of this color, as indicated crudely by the "red:green ratio," is almost identical in the two.

The reasons for this slight excess of "white" and the corresponding decrease in "black," on the part of the experimental series, can not be stated with any certainty. The difference may have no biological significance whatever. In any case, it must be urged that such a change, if it actually occurred, was not in the direction of the local type, *gambeli*, but quite the reverse. For *P. m. gambeli*, like other residents of the mountains and coastal plain, is distinctly *darker* than the desert race, *sonoriensis*. (See Table V.)

In Table III are given the mean values for linear measurements of the body and some of its appendages, along with those for the relative width of the dorsal tail stripe (ratio to circumference of tail), and the depth of pigmentation of the soles of the feet, graded according to an arbitrary standard.¹⁹

It will be seen that the "transplanted" series comprises animals having a somewhat greater mean body length than those of the "wild" series. Since the maximum size is the same for the two lots, this is without doubt due to the fact that the latter one includes a certain proportion of immature mice, and does not represent an increase in the size of the captive animals.²¹ This mean difference in general size makes it necessary to reduce the measurements to a common standard, before comparing the length of the tail, foot and ear in the two series of mice. In Table IV are given the approximate values which would have been found if all our mice had

¹⁹ *Journal of Experimental Zoology*, April, 1920; October, 1923.

²¹ It is my practice, in measuring wild mice, to include animals having a body length of 80 millimeters or more. Under these conditions, it is inevitable that some immature individuals should be included. These smaller specimens are not, however, skinned.

TABLE III
VARIOUS MEASURED CHARACTERS OF P. M. SONORIENSIS (ACTUAL MEANS)²⁰

	No.	Body length		Tail length (abs.)		Foot length		Ear length		Tail stripe (%)		Foot pigmentation	
		Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Wild	140	88.99 ± .24	4.18	72.36 ± .31	5.33	♂ 19.91 ± .04 ♀ 19.58 ± .06	.57 .66	17.17 ± .04	.73	28.12 ± .24	4.11	.90 ± .07	.68
Trans-planted:	53	90.81 ± .35	3.77	69.36 ± .43	4.43	♂ 19.59 ± .06 ♀ 19.34 ± .08	.37 .68	17.17 ± .07	.68	24.85 ± .30	3.22	.77 ± .06	.60

²⁰ For certain characters, the number of available individuals was less than the total number indicated, owing to the injury of certain parts in some specimens (see p. 489). This fact accounts for certain minor discrepancies in the magnitude of the probable errors. The number of "wild" mice upon which the figures for foot pigmentation are based is 49, instead of 140. These are the same 49 individuals the skins of which were used in the color tests. Foot measurements are given separately for males and females, owing to the pronounced sexual difference in the length of the foot.

had a body length of 90 millimeters.²² The figures for the two other characters require no such correction, since these are more nearly independent of general size.

TABLE IV
CORRECTED VALUES OF TAIL, FOOT AND EAR IN *P. M. SONORIENSIS*

	Tail	Foot (♂)	Foot (♀)	Ear
Wild	73.09 ± .31	19.97 ± .04	19.56 ± .06	17.26 ± .04
Transplanted	68.82 ± .43	19.64 ± .06	19.17 ± .08	17.08 ± .07

From Tables III and IV it appears that the mean values for tail length, foot length (in both sexes) and width of tail stripe are significantly lower in the animals which have been reared for a number of generations at La Jolla. That these differences do not represent a modification of the characters concerned, as a result of changed climatic conditions, is, I think, equally plain. For (1), in each instance, the figure for the "transplanted" series of *sonoriensis* agrees less closely with that for the local race, *gambeli*, than does the figure for the "wild" series (*cf.*, Table V); and (2) the changes are in the direction of those to which all races (including *gambeli*) are subject, as the result of captivity, irrespective of climate.

Peromyscus maniculatus rubidus: Some preliminary observations on an earlier lot of these mice from Eureka have been discussed above. Because of the unsatisfactory nature of these results, a new stock was trapped near Carlotta, Humboldt County, in October, 1917. Carlotta lies about 20 miles from Eureka and is considerably further from the coast. In the wild generation, the Eureka and Carlotta series of mice showed no significant differences, except in the color of the pelage, which was appreciably paler, on the average, in the Carlotta animals. The latter were, nevertheless, much darker than the La Jolla race (*gambeli*).

²² See *Journal of Experimental Zoology*, April, 1920, p. 385.

TABLE V

MEAN VALUES FOR CERTAIN CHARACTERS IN LA JOLLA RACE OF *P. M. GAMBELI*
(CORRECTED WHERE NECESSARY)²³

Tail length	75.52 ± .27
Foot, ♂	20.02 ± .06
Foot, ♀	20.13 ± .04
Ear	17.80 ± .04
Tail stripe	32.34 ± .26
Foot pigmentation	1.88 ± .09
Black	83.47 ± .23
White	9.70 ± .13
Color	6.83 ± .16
Red : Green ratio	2.96 ± .04

Carlotta mice of the C_2 generation were transferred to the open pens in August, 1919, the stock having been previously kept in small cages within the "murarium." The treatment, subsequent to this transfer, was the same as that employed in the case of *sonoriensis* (p. 488). In the present case, the mice which were ultimately measured, with a few exceptions, represented a minimum of four generations born at La Jolla, and a maximum of six or more. The exceptions were 7 individuals (out of 62) which belonged to the C_3 generation.²⁴

In Table VI are given the color values for this series of "transplanted" mice, along with those of the parent stock from which they were derived. Only fully mature pelages are here included. The skins of the former series were prepared in October, those of the latter in September and October. Here, as in the case of *sonoriensis*, many skins give evidence of molting on the reverse side, although here, too, the appearance of the pelage is seldom materially affected by this circumstance. Of the 62 skins originally prepared, it was necessary to reject 21, owing chiefly to the presence of white hairs, following injury (see p. 489). Indeed, this condition may be detected, to some slight degree, in a large majority of the pelages.

²³ The figures for tail length to tail stripe, inclusive, are based upon 175 wild individuals; those for the remaining characters upon 52 cage-bred (C_2) individuals.

²⁴ These were used for body measurements, but not for skins.

TABLE VI

COLOR ANALYSIS OF *PEROMYSCUS MANICULATUS RUBIDUS*
(CARLOTTA STOCK)

	No.	Black		White		Color		Red : Green	
		Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Wild ²⁵	38	85.56 ± .13	1.17	8.77 ± .06	.52	5.67 ± .11	1.01	3.39 ± .07	.61
Trans-planted	41	86.23 ± .12	1.16	8.45 ± .07	.71	5.33 ± .09	.86	3.04 ± .04	.34

With respect to the first three values (black, white and color), it is plain that the agreement between the two is fairly close. It is also plain that the slight differences between the earlier and later series are not due to a modification of the latter in the direction of the local race. The "transplanted" lot actually average slightly darker than the wild. On the other hand, the change in the red:green ratio is in the direction of *gambeli* and *sonoriensis*.

Whatever the significance of this last fact may be (if it has any biological significance), the condition of these pelages as a whole surely does not justify the conclusion that the introduced race has been modified by local climatic conditions.

Table VII renders possible a comparison between the wild and transplanted series with respect to the various measured characters other than coat-color. Owing to the considerably greater average size of the latter animals, it is particularly important, in the present case, that certain of these mean values should be reduced to the same standard body length (p. 491). Table VIII gives these corrected values for characters which require such correction.

In the case of this race of mice, it is plain that the linear measurements, with the exception of that for tail length, are significantly greater in the "transplanted" series. The mean tail length is slightly though not sig-

²⁵ See footnote under Table II.

TABLE VII
VARIOUS MEASURED CHARACTERS OF P. M. RUBIDUS (ACTUAL MEANS)

	Body length		Tail length		Foot length		Ear length		Tail stripe (%)		Foot pigmentation	
	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Wild	90.07 ± .31	4.89	93.58 ± .39	5.97	$\left\{ \begin{array}{l} \text{♂ } 21.38 \pm .06 \\ \text{♀ } 21.17 \pm .07 \end{array} \right\}$	$\left\{ \begin{array}{l} .74 \\ .78 \end{array} \right\}$	17.27 ± .03	.88	41.53 ± .34	5.17	2.22 ± .10	.87
Trans-planted	97.53 ± .36	4.27	97.86 ± .54	5.32	$\left\{ \begin{array}{l} \text{♂ } 22.27 \pm .08 \\ \text{♀ } 21.94 \pm .07 \end{array} \right\}$	$\left\{ \begin{array}{l} .61 \\ .54 \end{array} \right\}$	18.13 ± .06	.66	48.21 ± .52	5.64	2.29 ± .07	.81

²⁶ See footnote under Table III. The figure for foot pigmentation, in the wild series, is based upon the same specimens (38) which were skinned. A considerably higher mean value is obtained when the entire lot, containing many immature individuals, are included. The feet are more heavily pigmented in young animals.

TABLE VIII
CORRECTED VALUES FOR TAIL, FOOT AND EAR IN P. M. RUBIDUS

	Tail	Foot (♂)	Foot (♀)	Ear
Wild	93.72 ± .39	21.52 ± .06	21.08 ± .07	17.23 ± .05
Transplanted	92.67 ± .54	21.89 ± .08	21.74 ± .07	17.76 ± .06

nificantly less in the latter series, while the depth of foot pigmentation is approximately equal in the two.²⁷

When it is remembered that the Carlotta race considerably exceeds the La Jolla race in the length of the tail and foot, the width of the tail stripe and the depth of the foot pigmentation, it becomes evident that four or more generations of life at La Jolla have not modified the former race preponderantly in the direction of the latter. The single significant change in this direction (ear length) does not outweigh the greater changes in the opposite direction, particularly since ear length is the least characteristic of these racial differences.

In comparing the "wild" with the "transplanted" series of each race, certain statistically significant differences have been discovered, both in the case of *sonoriensis* and *rubidus*. It has been found, however, that these differences have not been preponderantly in the direction of agreement with the local race (*gambeli*), but rather in the contrary direction. Since, in respect to most of the characters here considered, *gambeli* is intermediate between the other two races, an inevitable consequence of these changes has been some degree of divergence between *sonoriensis* and *rubidus*.

A comparison of Tables II, III and IV with Tables VI, VII and VIII is instructive in this connection. It will be seen that in respect to all the characters under consideration, with a single exception, the transplanted series of *sonoriensis* and *rubidus* differ more widely from one another than do the wild series.²⁸ In the single case of the red:green ratio, the difference appears to be significantly less in the transplanted series.

It is well to explain at this point that I do not believe that the two introduced races of mice have actually become more unlike as a result of life at La Jolla. At least two explanations of this apparent divergence suggest themselves. In the first place, it is partly accounted

²⁷ See footnote under Table VII.

²⁸ All these characters showed a considerable initial difference, with the exception of ear length.

for by the greater modification (shortened appendages, etc.) which the *sonoriensis* stock has undergone as a result of captivity. On the other hand, the superior condition of the *rubidus* stock, at the close of the experiment (superior in some respects to that of the parent animals)²⁹ may have been the result of the survival of the sturdier strains in the course of four years' competition in the open pens.

As has already been pointed out, subspecific differences such as have been dealt with in the present paper have been attributed by some naturalists to local differences of environment, acting during the individual lifetime, or at most during a small number of generations. It is true that the environment in which these mice have been reared during the course of these experiments does not agree with the normal habitat of *P. m. gambeli* in the vicinity of La Jolla. Nor is the food by any means the same as that upon which they would subsist in nature, either here or elsewhere. These facts, however, are quite irrelevant to the present discussion. If any such prompt response to physical conditions actually occurred, we might reasonably look for a convergence of characters after the transfer of two geographic races to a common environment, even though that environment were a highly artificial one. That no such convergence has been detected during the period covered by these experiments is evidence of a greater degree of stability on the part of these characters than some writers have been disposed to admit.

The experiments have not, of course, established the probability that no such convergence would occur in the course of a vastly greater period of time, or under the influence of more extreme changes of environment. But they do create the presumption that mammalian subspecies in general,³⁰ and perhaps also of those birds,

²⁹ Not only the mean size, but the maximum size was considerably increased for both sexes.

³⁰ We have confirmatory evidence for one other species of *Peromyscus*, *P. eremicus*, the desert race of which has retained its original coat-color after several generations at La Jolla (Huestis, *Proc. Nat. Acad. Sci.*, October, 1923; Sumner and Huestis, *Biological Bulletin*, in press).

would show an equal degree of stability if their habitats were interchanged.

ARTIFICIALLY MODIFIED ATMOSPHERE

After it seemed probable that a simple transfer of these mice from one climatic region to another would fail to produce any appreciable modifications in their color, the possibility remained that positive results might follow the application of more extreme changes in the environment. Various distributional data, together with a meager amount of experimental evidence,³¹ pointed to atmospheric humidity as a factor of probable influence in relation to the coat-color of mammals and birds.

Accordingly, a "desert room" was installed, with the object of reducing the relative humidity of the atmosphere to the lowest point practicable. This result was effected in two ways: (1) by warming the air of the experimental room, thus insuring a reduction in relative though not in absolute humidity; and (2) by circulating the air by means of an electric fan over a series of trays containing anhydrous calcium chloride, thus extracting water vapor from the atmosphere.³²

The mean air temperature of the experimental room was 25.3° C., the mean relative humidity being 33.2 per cent.³³ In the control room, the mean temperature was 16.3°, the mean relative humidity being 73.8. The differ-

³¹ Beebe, *Zoologica*, Vol. I, No. 1, September 25, 1907 (published by N. Y. Zool. Soc.); Bonhote, "Vigour and Heredity," London, 1915 (p. 86); Sundstroem, *Amer. Journ. Physiol.*, May, 1922 (p. 425-433). In each of these cases, animals were exposed to *high* humidity and an *increase* of pigmentation was believed to have resulted. Hollister's interesting observations upon cage-reared lions (Proc. U. S. Nat. Mus., vol. 53, June 1, 1917) may likewise be mentioned in this connection, though it is not certain that the darker color of these animals was due to the climatic changes involved.

³² Quicklime (CaO) was employed as a dehydrating agent during the first half of the experiment.

³³ These figures are based upon thermograph and hygrograph records which are probably not very accurate. Only the records for the second year of the experiment have been here employed. They are compared with the La Jolla ("murarium") figures given in Table I, which are based upon two earlier years. This procedure seems sufficiently accurate for present purposes.

ence in relative humidity was thus very great, and the effects of this difference were obvious in the shrinking and "checking" of the wooden cages and the dryness of the hay which was provided as nesting material.

On the other hand, the reduction in *absolute* humidity was not as great as would have been desirable, owing to the limited capacity of the dehydrating agents for absorbing water vapor. The daily rations contained considerable moist food, and the excretions of the animals were doubtless by no means negligible in contributing to the water content of the air. Thus the latter averaged about 7.6 grams per cubic meter, a figure three fourths as great as that for the outside air (10.2 grams). The corresponding figure for Victorville is 5.9 grams per cubic meter, representing a considerably lower absolute humidity than that of my dry-room. As compared with Victorville, however, the *relative* humidity of the latter was much lower, averaging 33.2 per cent., instead of 49.8. These figures, representing the drying capacity of the atmosphere, are doubtless more important physiologically than the absolute amounts of water present.

This necessity for maintaining a high temperature in order to lower the relative humidity of the air was unfortunate for the purposes of the experiment. The conditions were frequently inimical to the health of the animals, a circumstance which was reflected in the greatly reduced fertility of otherwise prolific strains. On several occasions the temperature passed the danger point, and considerable numbers of the mice were killed by the heat.

Owing to the somewhat conflicting evidence from these experiments, the results will be presented rather briefly. It is my hope that a really adequate dry-room, together with another in which the humidity may be maintained near the saturation point, will be available for further investigations along these lines.

Peromyscus maniculatus dubius: Mice of this subspecies³⁴ were used most extensively, owing to their rela-

³⁴ *P. m. dubius* is a native of the Coronado Islands and of certain other islands near the coast of Lower California.

tively high fertility and normal development under conditions of captivity, as well as their more limited variability in respect to coat-color. It was early found that specimens transferred to the dry room when well grown underwent no appreciable changes in coat-color. Thenceforth, considerable numbers were reared from birth in the experimental room, some of these representing a second generation born in the latter.

In the first (juvenile) pelage it was noted that these dry-room mice tended to be appreciably paler than control animals of the same age. Numerous comparisons were made between broods belonging to the two contrasted lots, the difference being nearly always, though not invariably, in the same direction.³⁵ None of these juvenile skins were prepared, however, since it seemed more important to rear the animals to maturity, and, in general, the comparisons here referred to do not rest upon a perfectly secure statistical basis.

In the second (post-juvenile) pelage this initial difference almost invariably disappeared. With further molts, there was an undoubted reversal of the original relations, so that the dry-room mice, at the time of maturity, actually averaged somewhat darker than the control lot. They were likewise less highly colored, being of a more nearly neutral gray.

Despite a heavy mortality from heat, about a month earlier, 46 specimens of *P. m. dubius*, among those reared in the dry-room, were living when the experiment was discontinued in October, 1923. To the number stated, all of which were skinned at this time, should be added 8 which had been skinned earlier. The mice here considered ranged from 4 to 13 months old, some of them thus being far from mature. In the control series are 77 skins, ranging from 3 to 18 months in age.

For the purposes of examination, these two sets of skins were arranged in parallel series upon a table, each

³⁵ It is worth noting that some of the conspicuous exceptions belonged to the second generation born in the dry room.

of these being graded, according to age. A careful comparison of the two contrasted series fully confirmed the impressions which had been derived from the living animals.³⁶ When animals of the same age were compared, the dry-room series averaged slightly but unmistakably darker, as well as less highly colored, than the controls. This difference was least marked among the younger animals, and most marked among the older ones.

Since, in the normal course of development, the pelage tends to become paler and more richly colored, with the attainment of full maturity, the condition of the dry-room individuals suggested a simple retardation of development. Indeed, in many cases it was observed that the molt of dry-room animals was less far advanced than that of animals of the same age living under more normal conditions. A careful inspection shows, however, that the difference between these two sets of skins is not due to any mere difference of pelage phase. Not only are dry-room animals darker than control animals of the same age, but the former are darker at 13 months than the latter at 7 months.

Gambeli, heterozygous for albinism: The possibility occurred to us that an agency might affect the pigment formation of an animal having the "color" factor in a simplex condition, even though it might be incapable of producing this effect in an animal which was homozygous for the factor in question. For the purpose of such a test, albino *gambeli* were mated with wild-type mice of the same subspecies. The number of parent animals here employed was very small, so that no evidence for an experimental change of color could have been regarded as decisive, even if any differences of this sort had manifested themselves in the two sets of offspring under comparison.

Fifteen skins are available, derived from specimens which were either born in the dry-room or transferred

³⁶ It was not thought worth while to resort to careful analysis by means of the tint photometer.

there early in life. These have been compared with 20 controls. In each series, most of the specimens range from over 7 months to about 10 months in age, two being considerably older than this. In the present case, as in the previous one, the two series were laid out for comparison, each being graded according to age.

One's first impression, on viewing these two lots of skins, would probably be that the dry-room lot was paler. But this difference is due to the presence in the dry-room series of three exceptionally pale specimens. These three specimens can not, unfortunately, be compared with control ones of the same parentage, since there are none of these available.

More instructive is the comparison of members of "split" broods, *i.e.*, ones which were divided in early life, half of the individuals being placed in the dry-room and half used as controls. Skins are available for five such broods, aggregating 17 mice. While considerable individual variation in color is manifest (due perhaps to genetic segregation), there is no preponderant tendency for one series to be darker than the other.

Rubidus and gambeli (wild type): Small numbers of mice belonging to these two races were reared in the dry-room and compared during life with individuals reared in the control room. No differences were detected which could reasonably be attributed to environmental conditions.

SUMMARY

(I) Mice belonging to the subspecies *Peromyscus maniculatus sonoriensis*, from the Mojave Desert, were reared for more than eight years at La Jolla, the resulting stock representing a minimum of seven and a maximum of twelve or more generations. During this period, they did not, in respect to any single measured character, undergo a modification in the direction of the La Jolla subspecies, *gambeli*. On the contrary, the mice of the later generations were in some regards less like *gambeli* than were their ancestors trapped in the desert.

(II) Mice belonging to the subspecies *P. m. rubidus*, from the northwest coast of California, were reared at La Jolla for six years, the resulting stock (with a very few exceptions) representing a minimum of four and a maximum of six or seven generations. Here again, the slight differences between the ancestral stock and its descendants were not such as to indicate a modification in the direction of the local race. As in the case of *sonoriensis*, the mice of the later generations of *rubidus* were, on the whole, less like *gambeli* than were their wild ancestors from Humboldt County.

(III) Comparing the two introduced strains, *sonoriensis* and *rubidus*, there was no tendency towards convergence, under the influence of a common environment. To judge from the samples at our disposal, there was actually a slight divergence in respect to all but one of characters which were measured.

(IV) The nature of these slight differences between the transplanted and ancestral series of a given race renders it highly improbable that they have been due to changed climatic conditions. To some extent, they are known to be the results of captivity, irrespective of climate.

(V) Subjection of deer-mice of several strains to an atmosphere of high temperature and very low relative humidity gave conflicting results. In *P. maniculatus dubius* the dry-room animals were, on the whole, noticeably paler than the control, while in the gray juvenile pelage. This difference was not invariable, however, and the numbers were not sufficient to furnish decisive evidence of such a change. In any case, this initial difference in shade disappeared with the assumption of the second pelage, while the difference was actually reversed in later pelages, the dry-room animals now being somewhat darker. Such an effect, of course, was quite unexpected, in view of the prevailing pale coloration of desert mammals. But, in judging these results, the almost pathological character of the dry-room animals must be taken into account. In mice of certain other races, on

the contrary, no differences were noted between the experimental and control series, either in the juvenile or later pelages.

(VI) On the whole, one can not fail to be impressed by the comparative stability of these various races of mice under very marked alterations in the physical environment. As regards color characters, such almost wholly negative results are not in agreement with those of certain other experimenters who have reported pronounced color changes in animals, following considerable changes in atmospheric humidity. Nor do the present results afford any support for the view held by certain zoologists that the differences between geographic races or subspecies are purely "somatic" and therefore non-hereditary. Regarding the more difficult question whether climatic influences may not have a cumulative effect in the course of sufficiently great periods of time, our views must at present be decided by considerations of a taxonomic and distributional nature rather than by any available experimental evidence.

THE SEX CHROMOSOMES OF MAN¹

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RECENT studies in human spermatogenesis have cleared up the doubt which has so long existed over the number of chromosomes possessed by mankind. The somatic or diploid number for the female is 48, and this must be taken as the basic number for the race since the female is homozygous for sex. There is not complete agreement as yet about the full diploid number of the male, both 47 and 48 chromosomes having been reported.² On the other hand, a haploid number of 24 chromosomes has been found uniformly in males when fresh properly preserved material was used for study, and includes germinal tissue (testes) from Europeans (d'Winiwarter, '12), American Whites and Negroes (Painter, '23) and Japanese (Oguma and Kihara, '23).

In view of the small size of the human germ cell and the complexity of its chromosome make-up, it is really surprising that observers, using very diverse methods of preservation, should agree so closely in spermatogonial counts. If spermatogonial counts were the only avail-

¹ Contribution No. 182 from the Zoological Laboratory, University of Texas, Austin, Texas. In carrying out these studies on mammalian chromosomes the writer has been aided by a grant from the Committee for Research on Sex Problems, of the National Research Council.

² D'Winiwarter and Oguma and Kihara have reported 47 chromosomes. The writer is the only investigator who has published a paper in which 48 was shown to be the diploid number. However, Evans is reported by Babcock and Clausen to have counted 48 chromosomes ("Genetics in Relation to Agriculture," ed. 1918, page 538). And Conklin reports that Guyer has written him that he has also found 48 in recent studies ("Heredity and Environment," 4th ed. 1922, page 166). As yet, so far as I know, neither Evans nor Guyer have published on this subject, but Professor Evans very kindly has shown me drawings of spermatogonia in which there were 48 chromosomes, and Professor Guyer has indicated to the writer his intention of publishing on this subject in the near future.

able evidence for the somatic number, it is doubtful whether or not we could ever be certain which reported number was correct, 47 or 48. In taking issue with d'Winiwarter's count of 47, and placing the full diploid number at 48, the writer was influenced, not so much by spermatogonial counts, as by the conditions observed in the first maturation division. Here I found the same haploid number as d'Winiwarter, but instead of a single X chromosome, which d'Winiwarter had inferred was present (but not observed), I found an X-Y sex chromosome complex. If the sex chromosome is paired in the male, as I have reported it, then the diploid number must be 48 (46 autosomes + X + Y). If, on the other hand, a single X chromosome is present, as originally reported by d'Winiwarter, and very recently maintained by Oguma and Kihara, then the diploid number is 47 (46 autosomes + X). It will thus be seen that the real point at issue is the question of the type of sex chromosome carried by the male sex. In either event, of course, the full diploid number for the homozygous sex (female) would be 48 (46 autosomes + 2X).

In view of the facts stated above, the central point of interest in human spermatogenesis now shifts from the question of chromosome numbers to that of the type of sex chromosome carried by the male sex. As long as there is doubt upon this second question, the matter of the exact somatic number must remain in an uncertain status.

The general matter of sex chromosomes in mammals has engaged the attention of the writer for some five years, and during this period a number of different mammalian forms have been investigated, in order, first, to show that sex chromosomes do really exist in this group (a fact for which we had previously no very conclusive evidence), and second, to gain as much information as possible on the form and behavior of these elements during all phases of spermatogenesis. Since sending my human study to press, over three years ago, several other investigations have been completed (either in press or

have just appeared) which have proved illuminating for the human condition. Study IV is especially interesting because it deals with the sex chromosomes of monkeys, and I have been able to give crucial evidence for sex chromosomes of the X-Y type in these lower primates. The form and behavior of these elements are essentially the same as in man. New human material has also been studied, in which I have found the same conditions reported in my earlier human work. Finally, direct genetic evidence and other cytological work have appeared which point indubitably to the conclusion that man (the male) carries a Y chromosome.

The several investigations referred to above had been in press some time before the recent paper of Oguma and Kihara appeared. In view of the fact that the conclusions of these investigators, as regards the sex chromosome, run counter to my own recorded observations for man and closely related animals, it has seemed wise to discuss in a general paper the question of sex chromosomes in man. Here it is proposed to review the evidence for sex chromosomes in the work previously done by d'Winiwarter, Oguma and Kihara, and myself, to indicate the nature of the results of my second human study, just completed, and to bring together and briefly summarize such other cytological or genetic evidence as may be pertinent to the question before us.

At the outset, of course, one must consider what is crucial evidence for the presence of sex chromosomes in any animal. In a study about to appear (Study IV) I have discussed this matter at considerable length, account being taken of variations from the usual sex chromosome conditions and of those anomalies in ordinary chromosome form and behavior which may, under certain conditions, simulate sex chromosomes. We may, therefore, omit the details and exceptions and state that there are, in general, five cardinal points which should be obtained in order to be sure that a given chromosome (or complex) is really the element which determines sex. Assuming

that the male is the heterozygous sex, these are as follows: (a) Spermatogonial counts and a study of the morphology of the individual elements usually indicate the type of sex chromosome which will be found in maturation.³ That this point may not necessarily hold in lower mammals, at least, has been demonstrated recently by Agar ('23). In *Macropus*, the sex chromosomes sometimes fuse with autosomes, so that the spermatogonial number in the same individual appears to vary from 10 to 12 (the latter being the full diploid number). Under such conditions the observed diploid number would not be a reliable index of the type of sex chromosome. (b) The haploid count is needed as a check on the diploid number and when it is just half of the latter (for example diploid 48, haploid 24) indicates an X-Y sex chromosome. When the haploid is more than half the diploid, an X chromosome is indicated (47, 24). (c) The form and behavior of the sex chromosomes during maturation is the most direct and important evidence which one can obtain for sex chromosomes. If an unpaired X is present, it will be observed passing undivided to one pole in one of the maturation divisions. (In mammals the first is the reductional division for the sex chromosomes). Similarly, the X-Y elements, if such are present, may usually be identified as they segregate to opposite poles of the cell. If there has been an earlier association of the sex chromosomes with autosomes, it does not hold for this period. (d) Second maturation counts are needed to verify observation on the distribution of sex elements in the first division. (e) Finally, one must know the character of the female chromosome complex, in order to determine which is the X or female-producing chromosome.

PREVIOUS WORK

D'Winiwarter counted 47 chromosomes in spermatogonia and 24 in the first maturation division. Secondary

³ Thus an odd number, 47 for example, is suggestive of the X-O condition, or an even number (48) indicative of the X-Y type of sex chromosome. The form or morphology of the individual chromosomes should be studied, and homologous elements mated up (paired). When this last is done, chromosomes without mates of like size or shape can be identified.

spermatocytes showed either 23 or 24 chromosomes; hence he inferred that an unpaired X had passed undivided to one pole in the first maturation division. He did not identify this X chromosome. He found 48 chromosomes in the female.

Painter observed a diploid number of 48, and on pairing these up found that two were without mates of like size or shape. The haploid number was given as 24. In the first maturation division all elements appeared as tetrads except one, which was made up of two very unequal parts. These parts corresponded approximately in size to the unpaired chromosomes in spermatogonia, and were interpreted as X-Y sex chromosomes. The X and Y components were shown to segregate to opposite poles of the first maturation spindle. Second maturation counts were not made and the female condition was not investigated because of a lack of material.

Oguma and Kihara report a diploid number of 47. These were lined up and found to be paired except for the largest chromosome which they identified as the X. Twenty-four chromosomes were observed in the first maturation division, the largest element being again identified as the X. Its subsequent form and behavior was not followed.

At the time d'Winiwarter did his splendid work, the central point of interest was the question of the number of chromosomes characteristic of man, and questions of chromosome morphology and sex chromosomes were of secondary importance. Consequently, we find that the evidence which d'Winiwarter has given for sex chromosomes is what may be inferred from the numbers he observed in spermatogonia and in secondary spermatocytes. He never identified any particular chromosome as the X, nor did he observe it passing in an undivided state to one pole of the cell in maturation. His work may be harmonized with my own by assuming that he overlooked the very small Y in the second maturation division (see page 311 of Study II).

In my own work, when I found that d'Winiwarter's diploid count was approximately correct, I directed my attention primarily to the questions of chromosome morphology and behavior, especially during maturation, and the evidence which I have presented for the X-Y sex chromosome is based on direct observation of these elements as they segregated to opposite poles of the cell in the first maturation division. I lacked, however, the confirmation of second spermatocyte counts, and, of course, since I had not studied the female chromosomes, I could only infer (from conditions in the opossum) that the larger sex component was the X chromosome. This gap has been filled in part by my study of the lower primates, which will be referred to in detail later on.

The evidence for a sex chromosome of the X-O type which has been given by Oguma and Kihara rests mainly on spermatogonial evidence, the odd number (47) and the reported unpaired nature of the largest chromosome suggesting a single X chromosome in the male. Their evidence from the first maturation division can not be given much weight, because they did not follow the chromosomes beyond the metaphase, and did not show that any one of the 24 elements behaved as an X chromosome, *i.e.*, passed undivided to one pole. Furthermore, the chromosome which they have labeled "X" has the same sort of split which is shown in a number of other chromosomes in the same spindle. It is not clear why they call one element a single (unpaired) chromosome while the others are regarded as bivalents (tetrads). My own observations differ from these Japanese investigators in a number of points which will be taken up in the following section of this paper.

It is noteworthy that none of the investigators who have championed the X-O condition in man has observed the X chromosome passing undivided to one pole. The primary evidence for a sex chromosome of this type is found in the reported odd number of the spermatogonial chromosomes. On the other hand, I have been led to the X-Y interpretation because I observed in the first matu-

ration division the actual segregation of unequal sized components to opposite poles of the cell. The phenomenon was very closely similar to the condition observed in the opossum and was given the same interpretation.

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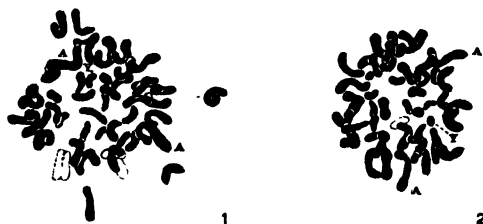
NEW OBSERVATIONS

At the time the paper of Oguma and Kihara appeared I was engaged in a study of new human material (negro), so that I at once carefully checked up the points on which they disagree with me. The excellent illustrations of these Japanese cytologists have enabled me to identify their X chromosome and to follow it through the crucial period of maturation. In the following section of this paper, I shall briefly review the facts which bear upon the points at issue between Oguma and Kihara and myself.

Spermatogonia: The observations of Oguma and Kihara and myself for this period differ in three respects; the total number of chromosomes (47 *versus* 48), the condition (unpaired or paired) of the largest spermatogonial chromosome, and the matter (absence or presence) of a very small Y chromosome. In the foregoing pages I have presented cogent reasons why we can not hope to settle this first point of difference by spermatogonial counts. There are always points in the chromosome complex where one has to interpret the structures observed. In all the figures which Oguma and Kihara have given, except figure 3, one may with reason count 48 or even 49 chromosomes. Furthermore, and this applies to the third point of difference also, it is very possible that one or both of the sex chromosomes have a tendency to associate with some autosome during spermatogonial division, as in marsupials. This tendency may be more pronounced in some human races than in others. I have observed and figured spermatogonial cells in which I could find only 47 chromosomes, the missing element being the Y. I have interpreted such cells as cases where the Y was hidden by some overlying element, but it may well have been a case of association.

The second point of difference has reference to the largest chromosome. I have shown that it is paired, and a study of d'Winiwarter figures 15 and 16 seems to indicate the same condition as I pointed out in Study II. Oguma and Kihara maintain that it is unpaired, and have interpreted it as the X chromosome. Their figures show this element, however, as being very slightly if at all larger than several other chromosomes in the cell, and if we keep in mind the error due to foreshortening, it would appear that their identification of the X rests on very insecure ground.

Oguma and Kihara dismiss the matter of the Y chromosome with the observation that they found the smallest chromosome paired. An examination of my own figures (Figs. 31 to 41, for example) will show that I observed in addition to the Y a pair of very small chromosomes which approach the Y in size.



FIGS. 1 and 2. Spermatogonial chromosomes of negro. Forty-eight elements in each cell. The largest pair of chromosomes labeled 'a'. 'Y' is the small "male producing" chromosome.

Figures 1 and 2 show the typical appearance of spermatogonial chromosomes in my new negro material. Forty-eight chromosomes will be found in both of these cells, and the two largest chromosomes are labeled a. The number of apparent chromosomes does not always total 48; in some cases I have found 47 or even 46 elements, while in others, there were 49 elements. In no cell have I found that one chromosome was noticeably larger than the rest of the elements, though, of course, the form of the two largest chromosomes was not always the same. The smallest chromosome which I would interpret as the Y is so labeled in the figures.

Growth Period: In insects, as is well known, the sex chromosomes retain the condensed form during the so-called growth period of primary spermatocytes, and appear as densely staining masses which we shall call chromatin-nucleoli. Similar structures have been observed in mammalian spermatogenesis, and since Oguma and Kihara use this evidence to support their claim for a large X chromosome, we must consider the nucleolar history in some detail.

Although it has been quite generally assumed that the chromatin-nucleolus in mammals was made up of the sex chromosome material, definite proof of this has only recently been forthcoming. In Study III, the writer has given a very detailed account of the behavior and fate of the chromatin-nucleolus of the opossum, in which it was shown that it is really made up of the X and Y sex chromosome material. Its behavior, however, is different from similar structures in invertebrates in a number of minor particulars, notably in this, that the X and Y components unite in the early pachytene stage into a single mass which has a very labile form during late pachytene and diplotene stages. At no time during this relatively long period, when the chromatin-nucleolus is large and is the most conspicuous element in the cell, do we gain the least hint either of the form of the sex elements or of their final size. In Fig. 3 A to G, I give again, with a descriptive legend, the history of the chromatin-nucleolus of the opossum, because, as I will show below, the chromatin-nucleolus of man behaves in just the same way.

In man (Fig. 4) the chromatin-nucleolus arises from an aggregation of chromatin knots which lie on the polar side of the nucleus. These unite into a large more or less oblong structure which presents many different forms during the late pachytene and diplotene stages (Figs. 5 and 6). If one were to judge the size of the sex chromosome by the size of the chromatin-nucleolus during this period, as Oguma and Kihara have done, he would conclude with them that it was a very large structure. As

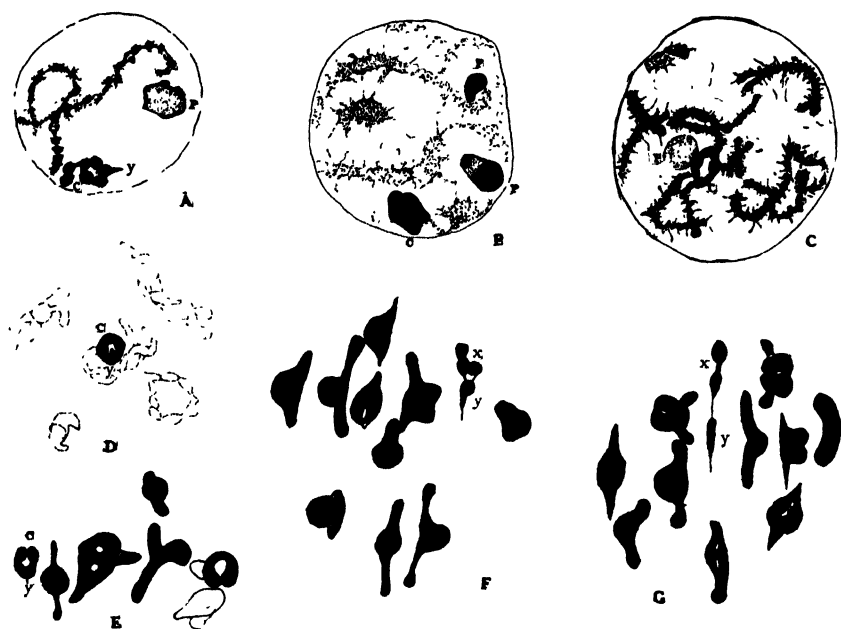
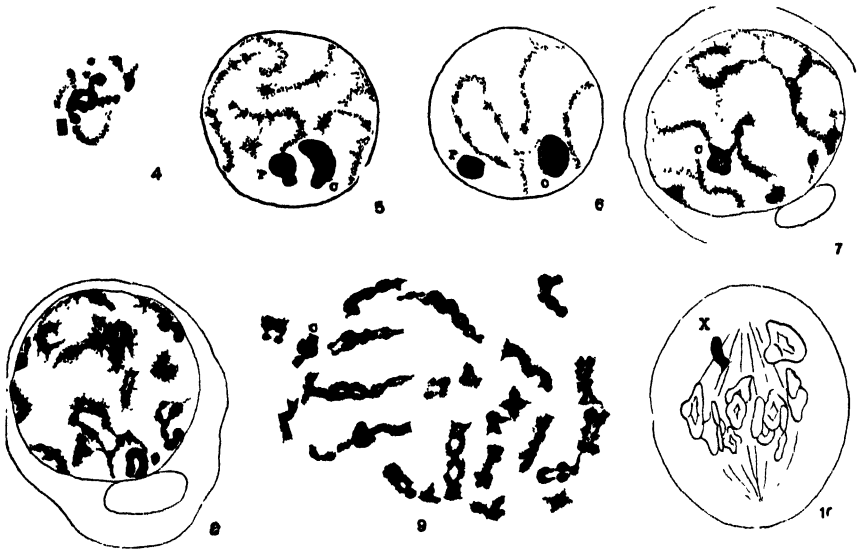


FIG. 3A to G. Showing the history of the chromatin-nucleolus 'c' in the opossum. A shows the formation of the nucleolus from knots of chromatin, the 'Y' element being labeled. B, late pachytene stage showing large size of chromatin-nucleolus 'c' and two plasmosomes 'p'. C and D are diakinesis stages; note reduction in size of chromatin-nucleolus. E to G show the way in which the chromatin-nucleolus forms the X and Y sex chromosomes.

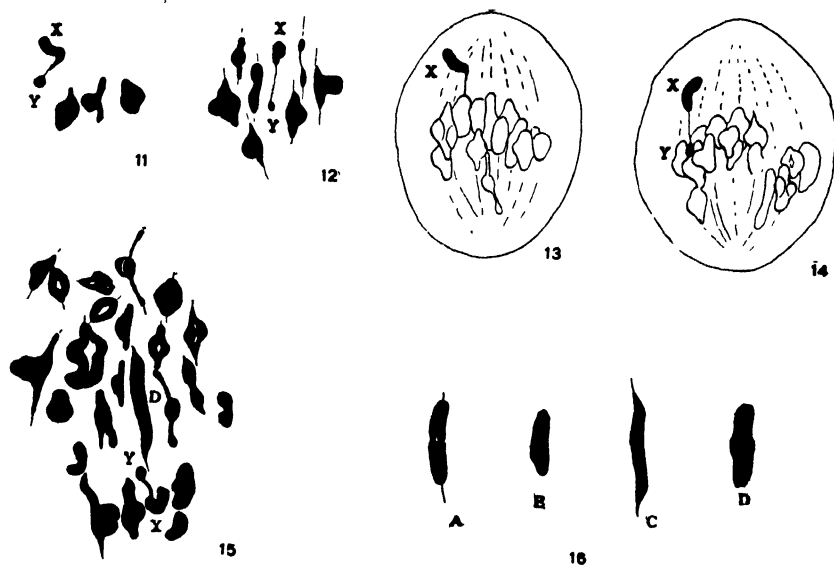
division approaches, however, the size of the chromatin-nucleolus decreases, probably by condensation and, perhaps, the loss of achromatic material, until by the early prophase it is a small structure (Fig. 7, "confused stage" and Fig. 8, early diakinesis). Note especially that in figure 8 the chromatin-nucleolus consists of a curved rod and a rounded element. Figure 9 is a late diakinesis stage which follows quickly after the condition shown in figure 8. (The chromosomes in this case have been drawn out separately, in order to show their form.) There are 24 elements present in this cell (from one section) which is the full haploid number. The chromatin-nucleolus is probably the structure labeled c, though I can not be sure in this. On the other hand, the longer chromosomes all consist of pairs of threads which are twisted together, this being of course the normal behavior of autosomes



Showing the history of the chromatin-nucleolus in man (negro). Only a small portion of the spireme threads are drawn. FIG. 4. Early pachytene stage showing formation of chromatin-nucleolus 'c' from a number of chromatin-knots. FIGS. 5 and 6 are late pachytene and diplotene stages showing large size of chromatin-nucleolus. 'P' are plasmosomes which disappear before spindle formation. FIG. 7, 'confused stage,' note small size of the chromatin-nucleolus 'c'. FIGS. 8 and 9, early and late diakinesis. FIG. 10, side view of first maturation division showing the true X chromosome. In this case the Y chromosome is not shown.

(tetrads) at this time. That the chromatin-nucleolus should have formed any of these larger chromosomes is inconceivable. Figure 10 is a cell which lay adjacent to that from which figure 8 was taken. The chromosome which I have interpreted as the true X is seen passing undivided to one pole. The Y in this case is hidden by overlying tetrads, but the point of interest is this, that the size of this X is about the same as that of the larger component of the chromatin-nucleolus (Fig. 8) from which it is undoubtedly derived.

Oguma and Kihara have used the chromatin-nucleolus at about the stage of figures 5 or 6, as evidence for a large X chromosome. The later history of this structure—which is quite similar to what occurs in the opossum—demonstrates, however, that it contracts and that by the time of diakinesis it must be considered as being among



FIGS. 11 to 12 show morphology of X-Y sex chromosomes of man. FIGS. 13 and 14, side views of first maturation division. The Y chromosome is not observed in figure 13, but note the heavy strand running from the X. FIG. 15 a 'spindle dissection' showing the 24 haploid chromosomes of man. FIG. 16A to D shows typical form of chromosome D, which is probably the element which Oguma and Kihara have identified as the 'X' chromosome.

the smaller third of the chromosomes. Figure 9 brings out the additional fact that all the larger chromosomes are made up of pairs of twisted threads. This is the most convincing sort of proof that all the larger spermatogonial chromosomes are paired.

First Maturation Division: The detailed study which I have made on new material for this stage has confirmed my earlier work. There are 24 elements in the spindle, 23 of which have the usual form exhibited by mammalian tetrads. One element is made up of two components of very unequal size (Fig. 11) connected together by a heavy chromatin strand. This is the X-Y sex chromosome complex which I found in two other individuals. Under normal conditions, the X-Y complex, being among the smaller chromosomes, occupies a position near the middle of the spindle, and can not be identified in side view unless the elements have already begun to segregate to opposite poles of the cell, or the spindle has been cut

so as to expose them to view. Figures 11 and 12 are cases of the latter sort in which both the X and Y are seen still connected by a heavy thread. As a usual thing in general side views of spindles one only sees the X going early to one pole, but it is always observed to be connected to the Y, which is frequently hidden in the equatorial plate, by a heavy chromatic thread (Figs. 10, 13 and 14).

Figure 15 is a spindle dissection of one of those rare cases where all 24 elements could be made out in a side view of the spindle. The X and Y elements are easily identified. All the larger elements in the first maturation division are tetrads, as was to be expected from the conditions observed in figure 9. The element which Oguma and Kihara have identified as an X is, as I interpret their figures, chromosome D of my Study II. It may be observed in figure 15, and it is noteworthy that it is drawn out at both ends by spindle fiber attachments. In figure 16, A to D, I give other characteristic forms of this chromosome. It is usually observed as a blunt heavy rod, often with a distinct split, as Oguma and Kihara have described. In favorable cells, however, its tetrad nature is revealed, as in figure 16D.

Anaphase stages of the first maturation division (Fig. 17) bear out the conclusions stated above. Figure 17 is

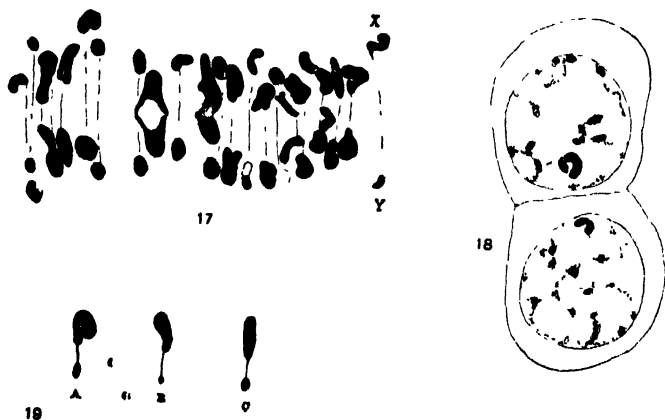


FIG. 17. Late anaphase of first maturation division showing the division of chromosome D. FIG. 18, resting stage of secondary spermatocyte. FIG. 19A to C shows the morphology of the X-Y sex chromosomes in primates. 19A, Brown Cebus; 19B, *Macacus rhesus*, and 19C, negro.

especially illuminating, as it shows chromosome D in the act of division. In this case the chromosomes have been separated to show their form. One may count 23 elements at each pole of the cell. I have been unable to find the two parts of chromosome W, which is the smallest tetrad in the human spermatocyte. At the top of this figure the X chromosome is observed, while its mate, the Y, is on the lower side. It is not always possible in late anaphase and telophase stages to count the full haploid number at each pole of the cell, but enough of these stages have been studied to show that, as in figure 17, all the larger chromosomes divide. If a very large X chromosome, such as described by Oguma and Kihara, had passed undivided to one pole of the cell, it could hardly be overlooked because of its size.

Second Maturation Division: So far the only evidence which I have to present for this period is that found in the rest stage of the interkinesis period just before the second maturation division. In figure 18 the two daughter cells of a first spermatocyte are shown. In one cell there is a large chromatin-nucleolus, presumably the X, while in the other daughter there is a much smaller Y.

Many secondary spermatocyte divisions have been studied, but in all cases so far, there has been marked irregularity in the time at which the individual chromosomes divide. This has precluded the possibility of decisive counts, such as are needed to prove that 24 chromosomes are always present in these cells.

OTHER EVIDENCE

In addition to the evidence which is found in a study of human spermatogenesis, the conclusion that man possesses the X-Y type of sex chromosomes is further supported by the fact that such sex chromosomes are found in the lower primates and in a number of other mammals.

Sex Chromosomes of Monkeys: The lower primates, of course, have a more direct bearing upon the sex chromosome condition of man than any other form. The fact is, that I was led to a study of the chromosomes of monkeys

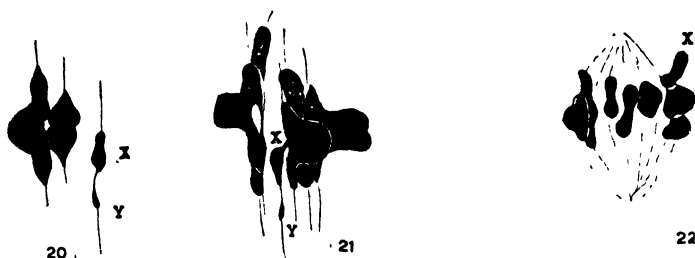
because it seemed probable that if the structures which I identified in man really had the fundamental function which I attributed to them, similar elements would be found in other primates. Preliminary reports covering both new world and old world forms have been published (Painter, 22b and 23b), and the completed work (Study IV) will probably have appeared by the time this article is printed.

Both the new world and old world monkeys show in their spermatogenesis the same sort of condition which I found in man. The spermatogonial number is even, and when the individual elements are paired up, two remain without mates of like size or shape. Among the tetrads, during the first maturation division, one element is found which is made up of two unequal parts. Figure 19A shows the form of this in a new world monkey (*Brown cebus*), 19B the condition in the *Macaccus rhesus*, and for ready comparison, figure 19C the condition in man. The similarity of the elements is obvious from figure 19, and their behavior in maturation is identical. In the case of the *Macaccus rhesus*, the somatic chromosome complexes of both male and female embryos were also studied. These confirmed the observations made in spermatogenesis. Forty-eight chromosomes (spermatogonial number) were found in both males and females. These two sexes differ in this respect, that while the male possesses two chromosomes which have no mates of like size or shape (a medium sized rod and a very small ball) the chromosomes of the female are all paired. In other words, the female carries two X chromosomes, while the male has an X and a Y. This work is of further interest as it shows that the larger of the two sex components is the female-producing or X chromosome, the smaller being the Y.

No more striking confirmation could be asked for than that given by the lower primates. Here the evidence for sex chromosomes of the X-Y type is complete, and the close similarity of both the form and behavior of the X and Y components, in man and in the monkeys, makes it

apparent that we are dealing with elements which must have a fundamental significance in all primates.

Sex Chromosomes in Marsupials: The opossum was the first mammalian form for which complete crucial evidence for sex chromosomes was given (Painter, '21), the X-Y type of sex chromosomes being demonstrated. Very recently Agar ('23) and Greenwood ('23) have given the results of their studies for five different Australian marsupials. In each of the species studied—and in most of these cases the female chromosome complex is also described—there is an X-Y sex chromosome complex. It may be pointed out further that, as in the case of the opossum, these sex elements were the smallest chromosomes in the cell. In figures 21 and 22 the X-Y sex chromosomes of *Macropus* are shown. (Taken from material kindly sent the writer by Professor W. F. Agar.)



FIGS. 20 and 21, showing X-Y sex chromosomes of *Macropus ualabatus*.
FIG. 22 shows the X chromosome in the albino rat.

Sex Chromosomes of the Horse: In Study V, now in press, the writer has described, for the horse, an X-Y type of sex chromosome. The evidence was not as complete as for the forms cited above, but a typical X-Y chromosome was observed in the first maturation division quite similar in form and behavior to that found in the opossum and in the primates.

In all the cases so far cited, X-Y sex chromosomes have been found, and in each case the X has been a relatively small chromosome and the Y component an exceedingly minute element. It would not be surprising, therefore, if in some mammals we should find that the Y had been lost. The rat appears to be an animal in which this has occurred. Allen ('18)) in his spermatogenesis of the rat

shows that only the X is present. In this case, however, the X is not the largest chromosome, but is a medium sized structure. In figure 23, a drawing of the sex chromosome of the rat is given (this cell was found in a slide presented to the writer by Dr. Ezra Allen). The X chromosome of the rat has proportionally about the same size, as in the case of the primates.

Genetic Evidence: The presence of sex-linked characters in man, such as color blindness, haemophilia, etc., have been known for a long time and have shown that the male sex carries one X chromosome, *i.e.*, is heterozygous for sex. It is only comparatively recently, however, that genetic evidence has been found which demonstrates that in certain vertebrates the Y chromosome may carry genes.⁴ The best known cases of this sort are found in teleosts, and have been carefully worked out by Schmidt ('20), Aida ('21), Winge ('22) and others. The only other vertebrate for which a similar condition has been reported is man, and this case rests upon one family history involving four generations described by Schofield ('21).

A father having a certain character (webbed toes) transmitted it to all his sons and none of his daughters. The sons, in turn, transmitted this character to all their sons and none of their daughters. The daughters from the several generations involved never showed the character, nor did they transmit it to any of their offspring. If the case has been correctly reported, as there is every reason to believe, the only possible explanation for this case is that the defect was carried by the Y chromosome of the male, as pointed out by Castle ('22).

Other cases of webbed toe inheritance have been reported in which the distribution of the defect did not show a sex chromosome transmission. Wright ('22) has pointed out, however, that the same outward expression of a character may be due to two entirely different genes,

⁴ In this respect the vertebrates differ from the invertebrates, because so far as we now know, the Y chromosome of invertebrates carries no genes.

and explains the different pedigrees which have been reported for webbed toes on this basis.

GENERAL CONCLUSIONS

In the foregoing pages a considerable volume of direct and indirect evidence has been presented which indicates that the sex chromosomes of man are of the X-Y type. It will scarcely be necessary to summarize this evidence here, but we must again emphasize the point that the cytological evidence is based upon direct observation on these elements during maturation. Those who have claimed that there was a single X chromosome in man have based their conclusion primarily upon chromosome counts in spermatogonia.⁵ The evidence for a single X chromosome is thus largely inferred, since none of the writers advocating this interpretation have observed it passing undivided to one pole of the cell during maturation.

Since all recent investigators on human spermatogenesis agree with d'Winiwarter that the haploid number is 24 chromosomes, it follows that the true diploid number for the male is 48 chromosomes as I have reported it (46 autosomes + X + Y). When 47 chromosomes are observed in spermatogonia, it probably means either that one element has been overlooked or else that one of the sex chromosomes is temporarily associated with some autosome, as is the case in certain of the lower mammals.

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⁵ In this paper I have not considered the earlier studies in human spermatogenesis. A full discussion of these works will be found in Study II beginning with page 310.

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GYNANDROMORPHS FROM X-RAYED MOTHERS

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IN the extensive work on *Drosophila* two kinds of individuals have been found which can not be classed as either male or female. Of one kind are the intersexes discovered and investigated by Bridges (1921). In an intersex the body as a whole shows a structure intermediate between that of the male and female. Of another kind are the gynandromorphs investigated in detail by Morgan and Bridges (1919). In a gynandromorph a part or parts of the body may be female in their structure and a part or parts male. The occurrence of both intersexes and gynandromorphs has been shown to be associated with a corresponding abnormal distribution of the chromosomes. Bridges (1921) has shown intersexes to be due to an abnormal proportion between the numbers of autosomal and X-chromosomes. Morgan and Bridges (1919) have collected a large body of evidence showing that the occurrence of gynandromorphs is due to non-disjunction or elimination of the X-chromosomes in some of the body cells during development.

The writer has already shown that X-ray treatment may induce abnormal distributions of the X-chromosomes at the time of maturation of the egg (Mavor, 1924). In the course of these experiments a number of gynandromorphs have occurred in the offspring of the X-rayed females and none in the controls. Although the number of gynandromorphs is small, it is believed their occurrence is evidence that X-ray treatment may induce the development of gynandromorphs.

To date in our X-ray experiments we have found four gynandromorphs among the F_1 of X-rayed females and none among the F_1 of control females. The first occur-

rence of gynandromorphs in our experiments has already been reported (1924). The two gynandromorphs occurred among the F_1 of X-rayed females in our first series of experiments. In this series of experiments wild type females were X-rayed for four minutes at 2.5 M.A. and 50 K.V. at a distance from the tungsten target of between 3.9 and 5.4 cm and mated to white-eyed males. According to our way of estimating dosage this dose is represented by 34.2–65.7 D. The total number of F_1 produced by the 22 control females was 7,340 red-eyed plus one exceptional white-eyed male. By the 19 fertile X-rayed females the number of F_1 produced was 2,883 red-eyed, 24 exceptional white-eyed males and 2 gynandromorphs. The two gynandromorphs were both of the bilateral type, one side being predominantly male and the other female. One of these, No. 1, is illustrated in

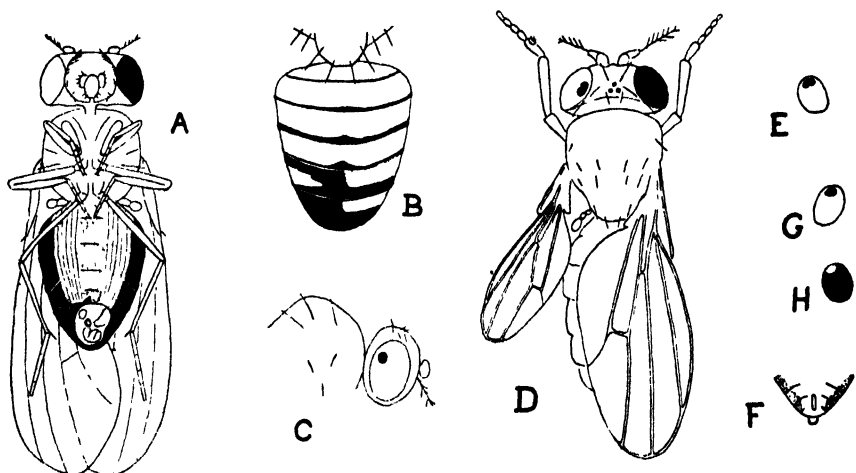


FIG. 1. Gynandromorphs from X-rayed mothers. A–C: Gynandromorph, No. 1, found in first series of experiments; A—ventral view; B—dorsal view of abdomen; C—right side of head. D–F: Gynandromorph, No. 5, found in a later experiment (VIth series); D—dorsal view; E—left eye; F—ventral view of posterior of abdomen. G, H: Gynandromorph, No. 6, found in the same experiment as No. 5; G—right eye; H—left eye.

Fig. 1, A, B and C. It arose as stated above from the mating of an X-rayed wild type mother by a white-eyed father. The right side was male, the left female. Presumably the maternal X-chromosome, the X-rayed

X-chromosome, became eliminated from some of the somatic cells, mostly those of the right side during early development. The second gynandromorph, No. 2, obtained in this series of experiments was essentially similar to the first.

Recently gynandromorphs have been again obtained among the F_1 of X-rayed females. In this case the females were X-rayed while in the pupa stage for ten hours with the current at 1 M.A. and 50 K.V. at a distance of 50 cm from the tungsten target. According to our method of recording dosage this is represented by 24D. The X-rayed females which were heterozygous for white and eosin eye-color, long and miniature wings, were mated to wild type males. The total number of F_1 produced in the 25 control matings was 9,627 regular males and females, four exceptional males and one exceptional female. In the case of the 107 females which were X-rayed the number of F_1 were 24,336 regular males and females, 118 exceptional males, 13 exceptional females and two gynandromorphs.

These two gynandromorphs were also of the bilateral type. One of them, No. 5, is illustrated in Fig. 1, D, E and F. Here the left side is predominantly male and the right female. Clearly the maternal chromosome carried white and miniature, being a crossover chromosome. The external genital organs were female (Fig. 1, F). The other gynandromorph which occurred in this experiment was predominantly male on the right side and female on the left. The right eye (Fig. 1, G) was white with a dorsal red spot. The left eye was red with a white spot corresponding in position and size with the red spot on the right eye (Fig. 1, H). A sex comb was present on the right fore leg and absent on the left. The wings were of approximately equal size. The external genital organs were male.

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It is to be noticed that the X-chromosome which was eliminated in the male parts of these two gynandromorphs was a paternal chromosome which had not been exposed to X-rays. Hence if these gynandromorphs were due to X-rays the action must have been indirect, the

X-rays having produced in the egg a condition which subsequently led to the elimination of an unexposed X-chromosome.

A large number of experiments have been performed in which females were X-rayed and no gynandromorphs appeared among their offspring. The technique of the X-ray treatment has been changed from time to time in the course of the investigation so that statistical treatment of the combined results is rather unsatisfactory. However, the only statistical comparison which seems justifiable is one involving the F_1 of all the X-rayed and all the control females. If the data of all our X-ray experiments be added together it is found that 373 females were X-rayed and produced 68,186 F_1 , of which four were gynandromorphs, while 237 control females (in each experiment the control females were sisters of the X-rayed) produced 65,128 F_1 , of which none were gynandromorphs. A formula developed by Karl Pearson (1907) for the probable error of the difference is particularly applicable to a case such as this since it takes into account the smallness of one of the classes, in this case the gynandromorphs. This formula gives 2.99, or approximately 3, as the difference between the number of gynandromorphs produced by X-rayed females and the mean expected of them from the number produced by the controls, divided by the probable error of the mean. Expressed in terms of probability, this gives a probability of approximately 20 to 1 that the difference is due to the treatment.

It should be mentioned that two other gynandromorphs have occurred in our laboratory—one was the F_2 of a control female, and the other occurred in connection with a classroom experiment. Since the data involving the occurrence of these is of quite a different nature from that given above they have not been included in the statistical treatment. It is to be noted that the occurrence of the gynandromorphs among the F_1 of X-rayed females, approximately 1 in 17,000, is not as frequent as gynandromorphs have been observed to occur in nature. Morgan and Bridges (1919) record a case where they were

found as frequently as 1 in 1,325. The evidence for X-rays acting as an agent inducing the development of gynandromorphs must rest entirely on a comparison of the behavior of control and X-rayed females genetically identical and reared under identical conditions. The fact that gynandromorphs have occurred more frequently under other cultural conditions than they have been found among the F_1 of X-rayed females merely shows that X-rays are not as effective an inducing agent as certain other unknown factors which may be present in cultures.

The occurrence of gynandromorphs as the result of X-ray treatment is of interest from more than one point of view. If the indications of these experiments should be substantiated by the further finding of gynandromorphs among the F_1 of X-rayed females the following conclusions would seem justified: (1) X-rays, in inducing the elimination of the X-chromosome, and probably in inducing non-disjunction, do not act directly on the chromosomes but rather by producing a condition which subsequently leads to the observed effect on the chromosome; (2) the Morgan-Bridges theory of the occurrence of gynandromorphs as due to the elimination or non-disjunction of the X-chromosomes during development, already well substantiated, would receive additional support if it is proved that a physical agent which is known to induce non-disjunction of the X-chromosome also induces the formation of gynandromorphs.

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COMMUNICATION BY SCENT IN THE HONEY-BEE—A THEORY¹

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A NUMBER of minute unicellular glands open upon the intersegmental membrane between the seventh and eighth terga of the abdomen of the honey-bee. These glands are called the glands of Nassanoff, after their discoverer. A number of suggestions have been made as to their possible use, but it remained for Sladen² to establish the fact that they are scent-producing organs. Sladen found that these organs give off a strong odor even when the part of the back to which they are attached is removed from the abdomen. He also asserted that this odor is the same as that which is given off when a number of bees are shaken to the ground before a hive.

Under these circumstances, as well as at the time of natural swarming, bees were known to produce a peculiar sound, sometimes called the "joyful hum." Sladen observed that this sound is produced by those individuals which first find the entrance to the hive, later by those next to them, and finally by others farther to the rear, so that soon all are informed of the location of the entrance into which they then make their way. He also observed that the odor mentioned above is emitted at this time and accordingly he asserted that it is the odor and not the sound which is the real means of information. He held, furthermore, that the sound is incidental to the special movement of the wings produced for the purpose of blowing the odor away from the body. He argued that we

¹ The author's thanks are due to Dr. E. F. Phillips, of the Bureau of Entomology, and to Dr. S. O. Mast for assistance in the preparation of the manuscript.

² Sladen, F. W. L., "A scent-producing organ in the abdomen of the worker of *Apis mellifica*," *Ent. Mag.*, Lond., Vol. 38, pp. 208-211.

have no evidence of an acute sense of hearing in bees, but that it is a well-established fact that they do possess a delicate sense of smell. Subsequent investigations have confirmed Sladen's observations, and his assertion that odor in this case is the real means of communication also is accepted generally.

Recently v. Frisch³ has ascertained that the use of this scent has a wider application. He asserts that when bees detect a new source of food they expose this gland and by fanning distribute the scent so that bees in the vicinity are attracted to the food. In this way sugar syrup or blossoms without odor, if discovered even by a few bees, are soon known to many gatherers. Park⁴ records an observation of similar behavior for bees carrying water, although in this case he states that the bees attracted were within a radius of eight or ten inches.

During the summer of 1923 the observations of v. Frisch and Park were confirmed by experiments conducted at the Bee Culture Laboratory of the Bureau of Entomology. Not only was it shown that bees attract others in their vicinity by emitting an odor while feeding, but it appears that the odor is a most important factor in enabling the discoverer of supplies to lead others directly to them. In other words, it appears that the discoverer of such supplies produces a scented trail through the air, thus enabling other bees to follow it. So fantastic is such a theory that one hesitates to announce it, were it not that the facts observed are of such a nature as well-nigh to establish such a theory as a fact.

Before proceeding to a consideration of such behavior in honey-bees, it is well to consider some of the conditions which must obtain in order that one animal may follow another by scent. Among such conditions are the following: (1) The creature followed must possess a scent; (2)

³ v. Frisch, Karl, "Über die 'Sprache' der Bienen," in München, Med. Wochenschr., 1920, pp. 566-569. Also in book form, 1923, Gustav Fischer, Jena, pp. 1-186.

⁴ Park, Wallace, "Communication among bees," in *American Bee Journal*, 1923, Vol. 63, p. 449.

this scent must be of such strength and character as to be perceptible to the individual which is following; (3) the scent must possess sufficient permanence to enable the pursuer to pick it up after the pursued has passed; (4) such a trail, although it may be broken, must possess a degree of continuity sufficient to enable the pursuer to cross such breaks. These points may now be considered with reference to the honey-bee.

That the honey-bee possesses a scent is generally accepted. Not only does it possess a body-scent, as do many other creatures, but it also possesses a special scent-producing organ, as previously noted. According to Sladen,² Shaftesbury⁵ and v. Frisch³ and the present writer's observations this gland produces scent of quantity and quality such as to be perceptible to man. This being true, we have the possibility of scent production in the bee of such quantities that considerable dilution may occur and yet sufficient strength be retained to be perceptible to another bee. It is well established that honey-bees have a far more acute olfactory sense than has man.

Regarding permanence of the scent trail, facts favor the present theory when contrasted with conditions under which other animals follow scent trails. In well-known cases of tracking by scent, the trail often remains for hours, as for example, in tracking by bloodhounds. In the case of the bee, however, permanence of trail need be a matter of minutes only, or even of seconds. Observations of the writer confirm the findings of other investigators who assert that bees which act upon the information received from the discoverer of a new source of supplies, do so promptly, that is, they usually leave the hive within a few seconds. Occasionally they defer the start for as much as two minutes (Park⁶). Such a delay, however, is about equal to the average time required by

⁵ Shaftesbury, A. D., "Some habits of honeybees," Thirteenth Annual Report of the Md. State Beekeepers Assn., 1922, pp. 11-22.

⁶ Park, Wallace, "The language of bees," in *American Bee Journal*, 1923, Vol. 63, p. 227.

a gatherer to deposit a load of nectar within the hive. Such a delay, accordingly, enables the associate gatherer to leave at approximately the same time as the discoverer. Whether the associate leaves promptly or whether it defers its departure until the discoverer leaves again, in either case it is able to pick up a fresh trail leading from or to the source of supplies, if such a trail exists.

If a trail possessing a permanence of from two to three minutes is all that is required to support the present theory, there remains to be considered the possibility of the trail having sufficient continuity to enable another bee to follow it. This point is supported by analogy, and for this purpose the observations of hunters may be cited. In hunting, game sometimes passes within clear view of the hunters so that its path may be seen. Not infrequently is it observed that the dogs run in a course parallel to that taken by the quarry and at a distance of a hundred or more yards to one side of it. So striking is this behavior that were it not explainable on a physical basis, one would be justified in the assumption that dogs are indifferent trailers. The apparent discrepancy is explained when it is realized that winds which are passing across the path of the quarry carry the scent to leeward and that the directing influence in this case is not actual footprints but is in reality a movable column of scented air connecting the starting point of the chase with the fleeing animal. Supporting evidence for the carriage of scent which is perceptible for great distances is also found in the fact that game is apprised by scent of the approach of hunters, while they are yet at distances of a half mile or more. Trails in the snow likewise have established the fact that certain carnivorous animals are attracted to their prey over equal distances. Such wind-borne scent is of such a nature as to enable the creature even to locate its prey.

In further consideration of a movable column of scented air it may be helpful to study the action of a similar column of visible gases. Such a column may be noted

along railroads on cool mornings, when the steam from passing locomotives lags behind in a long drawn-out column. This column, still maintaining its continuity, is frequently carried hundreds of yards by wind blowing across the course of the railroad. The visibility of this column is at length lost, but this is often due to the evaporation and precipitation of its particles rather than to the particles being scattered by the winds. A still better example, although a less common one, is that of sky-writing, which has been introduced recently. In this case the conditions are almost identical with those under which our hypothetical "tracer-bee" would have to work. In sky-writing an aeroplane is used, and from this smoke or visible gases are released; the machine being maneuvered at the same time so as to describe the letters or figures required. Such tracings persist to the extent that they may be carried by the winds for a mile or more. Not only do these tracings retain their visibility during this time, but the various parts of the individual letters retain their identity. This evidence shows that there is little tendency towards the breaking up of such columns of air. The effect of the winds is principally that of transportation of the column as a whole rather than a dissipation of its component parts. This being the case it is reasonable to suppose that a scent-laden column, although invisible, has equal or greater persistence than the visible column described.

It has been shown that columns of smoke persist for periods of several minutes, the length of such persistence being dependent upon atmospheric conditions. It has been shown also that columns of scented air likewise are known to persist for considerable periods. It now remains to examine the evidence tending to show that honey-bees produce a column of scented air which serves to direct a fellow-worker to the location of newly discovered supplies.

As already pointed out, Sladen shows that a scent is emitted and that it serves to attract other bees to the en-

trance of the hive. Von Frisch and Park assert that this scent is also used to attract bees over limited areas to the location of supplies of honey, nectar, sweetened water and pure water. All these observations have been confirmed by the present writer on many occasions. It is also apparent that the bees use this scent gland at times when they are not actually in contact with the supply of food.

In one set of experiments a maze was introduced at the entrance to the bait-box. Through this maze the bees at first were reluctant to pass, but under these circumstances it was observed that more than the usual amount of "fanning" was exhibited by bees which had gained entrance to the box. Not infrequently a bee would remain fanning for 30 or 40 seconds at the inner end of the maze, and during this fanning the scent gland was continuously exposed. During and shortly after such fanning, newcomers were directed more readily to and through the maze. This fanning often became so violent that the claws of the bee were insufficient to anchor it to the floor of the box, in which case the bee fell forward, often making a complete somersault. In righting itself it almost invariably came up with its scent gland still exposed. Occasionally, a bee was seen poised on the wing within the box and with the scent gland exposed.

In another set of experiments not more than one bee was allowed in the box at one time. It was observed that some of the excluded bees flying about the bait-box had their scent glands exposed. Finally, it was observed that the scent glands of some of the arriving bees were exposed, while the bees were yet on the wing and before they had alighted on the platform on which the bait-box had been placed.

These observations are significant, since they show that the scent gland is actually operated while the bee is on the wing and especially that arriving bees sometimes have their scent glands exposed. It may then be inferred that the scent glands are in operation continuously from

the time the bees left the vicinity of their hive. The difficulty attendant upon a check-up on this inference is great; since the bees fly at considerable height and at a rate which makes observations difficult if not impossible. The point that this particular behavior may at times be seen clearly when they do slow up in preparation for landing is a point which can not be ignored.

The theory of a scented trail is strengthened by the apparent elimination of other theories which have been proposed, that is, the sight theory and the theory of a general search. The theory that the hive-mate follows the discoverer by sight has been practically abandoned, since it is established that the hive-mate does not accompany the discoverer, but that it usually leaves the hive either before or after the discoverer and also that it frequently arrives at the bait after the discoverer has left for the hive. The theory of a general search for discovered supplies is weakened by the frequently recorded observation that at first only a few bees respond to information of such supplies by going to the fields. This fact discredits the belief that such bees search indiscriminately in all directions until by chance the supplies are found. If such conditions actually were to obtain, these two or three bees must search thoroughly more than five hundred acres of surrounding territory to locate a practically scentless bait of sugar syrup placed at a distance of one half mile from the hive. Not only would the first hive-mate be required to locate such supplies by chance, but each additional bee would likewise be required to find the supplies by chance. This condition would exist until a line of flying bees were established which could be followed by sight.

During the experiments at Washington it was observed that when two locations are desired at which to feed or bait bees from a particular hive, it is best to catch one or more bees from in front of the hive and carry them to the location desired. In no case was it found practicable to establish baits at various points and then introduce

bees at one of them, hoping that in a general search bees from the same hive would locate the other baits. Such a proceeding is, moreover, wholly at variance with the well-established practices of bee-hunters when establishing cross-lines.

In conclusion it should be stated that exposed scent glands were not observed on bees during their flight between the hive and the location of newly discovered supplies, except that exposed scent glands were observed on bees coming from the hive when they were within a few feet of the food supplies and after they had slowed down in preparation for landing. The inference may be made, however, that the discoverer does emit an odor from its scent gland throughout its passage between the supplies and its hive. It may be inferred, further, that such an odor enables a hive-mate to follow such a discoverer readily, and that it is in this manner that additional bees are led to the supplies.

VARIATIONS IN THE PREMAXILLARY OF *EURYCEA BISLINEATA*¹

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IN my study of skulls of large numbers of *Eurycea bislineata* in connection with a more extensive morphological problem, I have come across an occasional example of premaxillary which deviates from the usual fused type for this species (Figure 1, a and b) in that the two ascending processes are joined along their medial borders to a greater or lesser extent, thus completely inclosing a fontanelle. These examples (Figure 1, c, d and e) present in the more extreme cases an appearance quite different from the usual type and closely resembling that of *Pseudotriton ruber* (Figure 2, c) and *Desmognathus fusca*.

At first these cases seemed to me to have little significance, since from the widely separated position of the ascending processes in the typical larval stage there normally occurs, simultaneously with the structural changes leading to metamorphosis, a gradual approximation of these two processes so that they come to lie near the midline, and the united condition seemed to indicate only a slightly more extensive ossification in certain individual cases resulting in the fusion of the processes. When, however, younger individuals were found in which this same unusual type of premaxillary occurred, I was led to consider the possibility that this was a matter of deeper significance than a chance difference in the degree of ossification.

I have, therefore, examined recently all the individuals constituting two representative collections of larvae of *Eurycea bislineata* from a single locality, Bears' Den

¹ This paper is No. 117 of the Contributions from the Department of Zoology of Smith College.

Brook, on Mt. Toby, in Sunderland, Massachusetts. These collections, made in June and August, respectively, of 1916, comprised together a fairly complete series of 109 individuals ranging from recently hatched larvae of about 15 mm in length to metamorphic individuals probably from two to three years old and measuring from 50 to 60 mm. They had been preserved in formalin and subsequently stained *in toto* in alizarine for the study of bony structures, which are thus differentially stained. Each of these was examined by the simple process of stripping

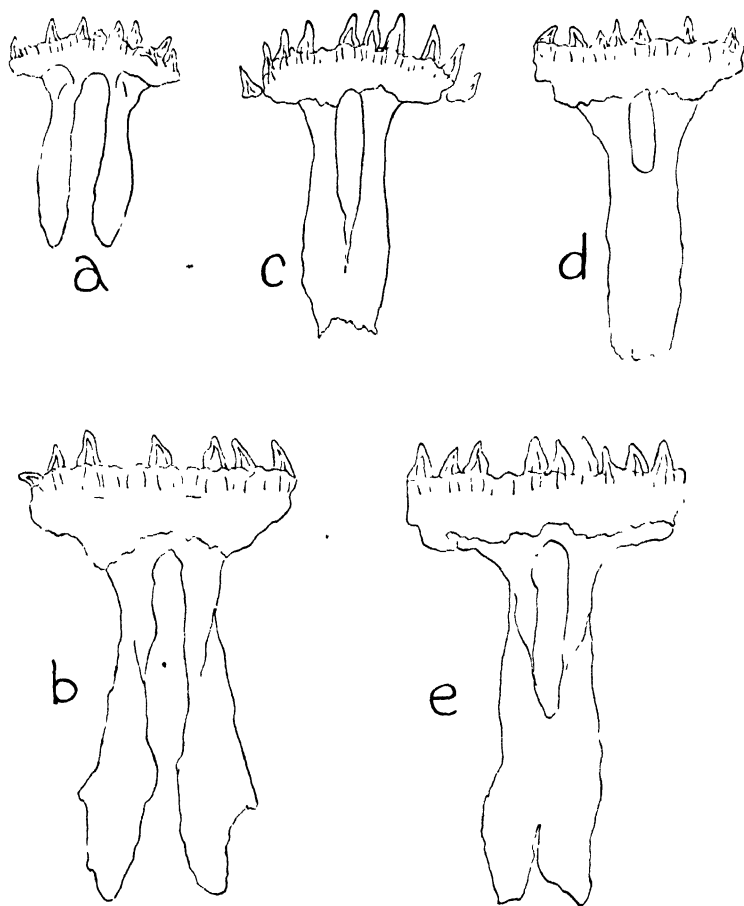


FIG. 1. Ventral views of premaxillaries of *Eurycea bislineata*. $\times 18$. Drawn from dissociated bones stained with alizarine. (a) Usual types as seen in a 27 mm larva; (b) Usual type as seen in an 81 mm adult male; (c) Unusual type found in a 44 mm larva; (d) Unusual type found in a 55.7 mm advanced metamorphic individual; (e) Unusual type found in a 67 mm young adult male.

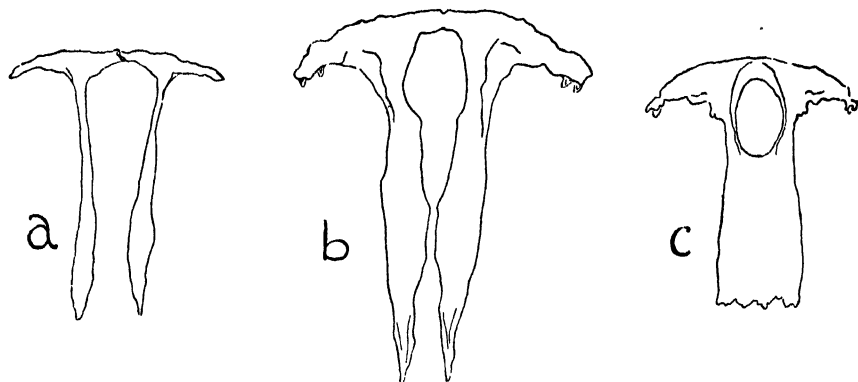


FIG. 2. Dorsal views of premaxillaries of (a) *Plethodon*, (b) *Gyrinophilus* (unusual type), and (c) *Pseudotriton*. $\times 18$. Drawn from *in toto* preparations stained in alizarine and cleared in glycerine, loaned by Dr. E. R. Dunn.

off the skin over the dorsal surface of the snout, thus revealing very satisfactorily against the white background of the surrounding tissues the whole dorsal aspect of the purplish-red premaxillary. Among the 109 specimens thus examined five (or 4.5 per cent.) were found to be of the unusual, more extensively fused type. These, together with controls of approximately the same size and developmental stage, were then further dissected to remove certain muscles and glands, and subjected to partial dissociation and clearing by means of caustic potash and glycerine, in order that the form and relationship of the premaxillary might be worked out and drawn more accurately. In all this work a binocular dissecting microscope was used, and the drawings were made by the use of a Zeichenokular, attached to this.

The five cases, which, together with the controls, are shown in Figure 3, comprise the following sizes and developmental stages:²

Typical larval male, length 22 mm.

Incipient premetamorphic female, length 37 mm.

Premetamorphic females, lengths, 46 mm, 51 mm, and 56.6 mm, respectively.

² Wilder, I. W., "The Relation of Growth to Metamorphosis in *Eurycea bislineata*," *Jour. Exp. Zool.*, Vol. 40, 1924.

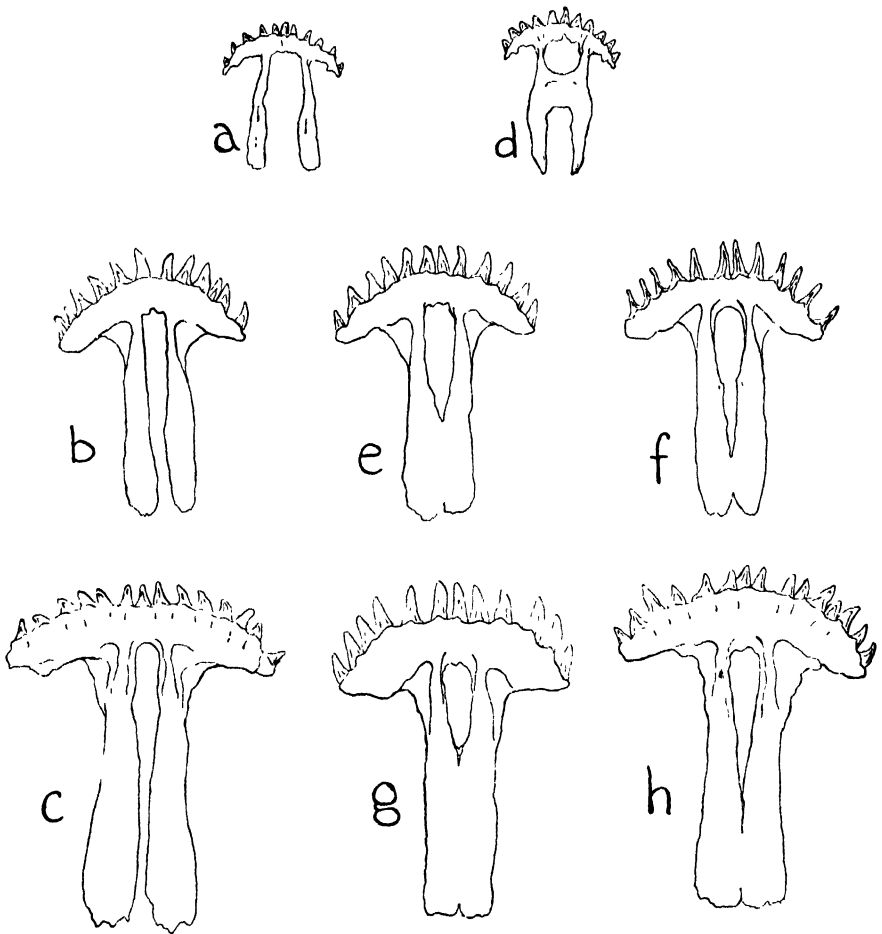


FIG. 3. Antero-dorsal views of premaxillaries of *Eurycea bislineata*. $\times 18$. Stained in alizarine and cleared in glycerine. Usual types: (a) 25 mm typical larval male; (b) 36 mm incipient premetamorphic male; (c) 51 mm premetamorphic female. Unusual type: (d) 22 mm typical larval male; (e) 37 mm incipient premetamorphic female; (f) 46 mm premetamorphic female; (g) 51 mm premetamorphic female; (m) 56.6 mm premetamorphic female.

Comparison of these cases shows a considerable degree of variation in the extent of union, which, however, does not seem to be correlated with the size or the developmental stage of the individual. This is particularly emphasized by the fact that the young larva shows one of the most conspicuously marked types of all, since in this stage, as is shown by the control, the ascending processes are normally so widely separated that their union across

the midline can by no possibility be attributed to a mere chance difference in the extent of ossification leading to the fusion of parts otherwise nearly in contact. That we are dealing here with a distinct variant is further indicated by the fact that the fusion of the ascending processes results in most cases in a somewhat narrower ascending region than that shown by the control. Attention should be called to the fact that examples of the unusual type have been found in both sexes and from two different localities, Western Massachusetts and Long Island, New York.

The premaxillary has never been given an important taxonomic value in the Caudata. While the Proteidae, Cryptobranchidae, Sirenidae, Hynobiidae and Ambystomidae show, so far as has been reported, the primitive paired or unfused type of premaxillary and the Amphiumidae the unpaired or fused type, in the large families of the Salamandridae and the Plethodontidae both types are present. In the latter family, for example, *Ensatina*, *Plethodon*, *Hemidactylium*, *Hydromantes* and *Gyrinophilus* are described as presenting the typical primitive paired form (Figure 2, a) while other genera such as *Ædipus*, *Eurycea*, *Pseudotriton*, *Batrachoseps* and *Desmognathus* show the unpaired type with varying degrees of fusion from a form like *Ædipus*, in which only the extreme anterior region is involved, to forms like *Pseudotriton* and *Desmognathus* in which the ascending processes are so completely fused as to leave only a fontanelle. It is evident from the data presented in this paper that *Eurycea bislineata* shows a range of variation of the premaxillary which includes these two extremes and thus covers differences which exist in this regard between different genera.

My colleague, Dr. E. R. Dunn, informs me that he has observed occasional cases of fusion of the ascending processes of the premaxillary in other species of *Eurycea*. He has also brought to my attention an equally significant variation which he has found in the premaxillary of *Gyrinophilus* (Figure 2, b). In this the simple fused

type is found instead of the paired type supposed to be characteristic of this genus. We have no data as to possible percentage of occurrence of this fused type in *Gyrinophilus*. This single case, however, emphasizes the probability of a similar variability in the premaxillary in any form, and should be a forcible warning against raising this bony element to the taxonomic importance which Noble³ gives it when he makes it one of the three distinguishing features of the genus (*Ædipus*: "(1) No prefrontal; (2) no septomaxillary; (3) premaxillae ankylosed only at their extreme anterior ends." The weight which Noble gives to the third point he further emphasizes in his statement, "*Ædipina*, which on external features one would consider nothing but an elongate *Ædipus*, differs radically from this genus in its fused premaxillae," apparently, moreover, basing his knowledge on a single specimen of *Ædipina* to which he refers as "the specimen of *Ædipina uniformis* which I dissected." However this may be, every taxonomist knows that in many instances only a single individual is available and that too often, even when there is an abundance of material at hand, descriptions are based upon either a single or at best a very few individuals.

If the premaxillary, which is one of the oldest and most constant of bones of the vertebrate skull, proves to be so variable within the limits of a single species, one is forced to believe that other bones might be found to be no less so, were a hundred individuals to be examined. Is it not possible that the *range of variation* of such a part may in itself be a matter of much greater significance as indicative of systematic relationships than a single type regarded as a constant character for the genus or species? From the standpoint of the morphologist, certainly, one would wish to urge the examination of larger numbers of individuals with regard to each detail of structure which is to be made use of, as a sound basis for descriptive and taxonomic work.

³ Noble, G. K., "The Anterior Cranial Elements of *Ædipus* and Certain other Salamanders," *Bull. of Am. Mus. of Nat. Hist.*, Vol. XLIV, 1921.

FURTHER STUDIES OF THE RELATIONSHIPS OF THE STRUCTURAL CHARACTERS OF MAMMALIAN HAIR¹

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SINCE the writer's preliminary studies of the structural characteristics of the hair-shafts of mammals were made,² continued observations have been throwing more and more light upon the relationships of the hair-shaft structures to one another, and to the groups of mammals in which they occur. In the earlier studies it was pointed out that the four structural units of the hair-shafts examined are susceptible of classification upon the basis of form.

The four structural elements of the mammalian hair-shaft (Fig. 1) are: (1) The medulla, or "pith" of the hair, made up of variously shaped and disposed cells or chambers, representing cornified epithelial elements; (2) the cortex, surrounding the medulla, composed of elongated, fusiform, often much-shrunken cells (sometimes referred to as the hair-spindles) coalesced into a rigid,

¹ The writer acknowledges, with gratitude, his indebtedness to the following, for sending him samples of hair: Dr. F. A. Lucas, the late Dr. J. A. Allen, Mr. L. R. Sullivan and Mr. H. Lang, of the American Museum of Natural History, in New York; Dr. H. D. Reed, of Cornell University; Dr. Aleš Hrdlička, Dr. G. S. Miller and Dr. N. M. Judd, of the United States National Museum in Washington; Dr. A. K. Fisher, of the Bureau of Biological Survey; Mr. C. G. Potts, of the United States Department of Agriculture; Dr. Chi Ping, of the Southeastern Teachers' College, Nanking, China; Dr. R. L. Ditmars and Dr. W. T. Hornaday, of the New York Zoological Gardens; Dr. F. R. Speck, of the University of Pennsylvania; the Metropolitan Museum of Art, in New York; Dr. T. C. Nelson, of Rutgers College, and Mr. M. W. Meek, of the Meek, Court Co.

² Hausman, L. A.: (1) "A micrological investigation of the definitive hair structure of the Monotremata," *Am. Jour. of Anat.*, Sept., 1920, p. 563; (2) "Structural characteristics of the hair of mammals," *AM. NAT.*, Vol. 54, 1920, p. 496; (3) "Mammal fur under the microscope," *Nat. Hist.*, Vol. 20, 1920, p. 434.

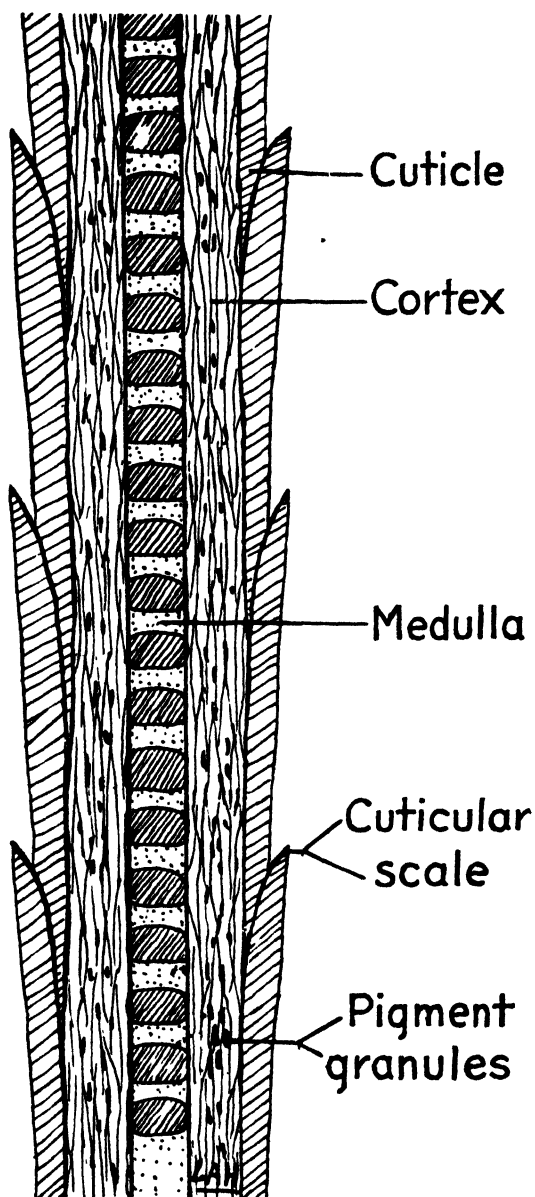


FIG. 1. Optical longitudinal section through a generalized mammal hair of the discontinuous medulla type.

almost homogeneous, hyaline mass; (3) the pigment granules, to which the color of the hair is primarily due (though in some hairs the pigment is diffuse). These granules are distributed within and among the hair-

spindles. Studies which have been³ and are now being made, of the disposition of the pigment granules, and especially of the patterns which they form in the hair-shaft, give earnest of some interesting correlative data. (4) The cuticle of the hair, which is its outermost integument and which is composed of thin, horny, transparent plates of a multitude of forms.

The cuticular scales of the hair are of two general kinds (Fig. 2), the imbricate and the coronal. Of the

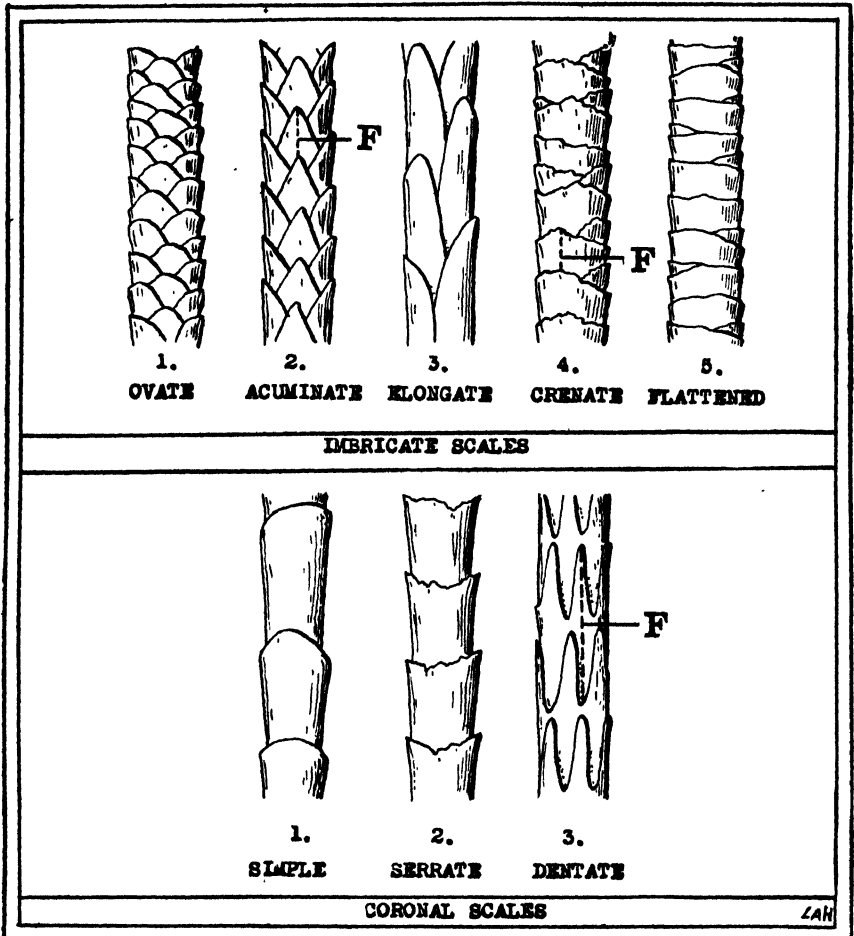


FIG. 2. The eight types of cuticular scales of mammalian hair. F shows how the proximo-distal diameters of the free surface of the scales are measured.

³ Hausman, L. A., "Hair coloration in animals," *Sci. Mon.*, Vol. 12, Mar., 1921, p. 215.

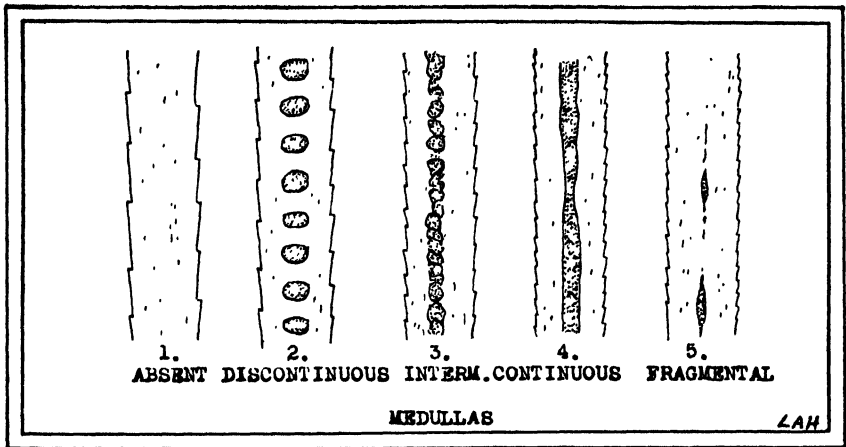


FIG. 3. The five types of medullas of mammalian hair.

former we have recognized five elemental varieties, *viz.*, ovate, acuminate, elongate, crenate and flattened. Of the latter there are three varieties, *viz.*, simple, serrate and dentate. All scale forms which have now been found in the hairs of mammals (in all over 370 species have been examined) are referable, as variations, to these eight categories.

The medullae lend themselves to classification thus (Fig. 3): (1) Absent altogether, (2) discontinuous, as when the medullary cells or chambers are separated, (3) intermediate, with the separate chambers beginning to fuse, (4) continuous, in which the cells are massed into a nearly uniform rod-like structure in the center of the hair-shaft, and (5) fragmental, in which case elongate fragments of the medulla are distributed irregularly along in the axis of the shaft.

In the discussion of the scale and medulla characteristics of hairs which follows, the status of these structures is considered midway between the base and the tip of the hair. The hairs used for comparison were the under or fur-hairs of the specimens, taken from the region of the middle of the dorsum. It was found that among the *Primates*, below the *Hominidae*, the under hair from the dorsum was identical in structure with that of the head.

The nature of the relationship between the hair structure and hair-shaft itself was first suggested to the writer following the examination of the hairs of sixteen species of *Primates*, chosen at random and representing the nine families and ten subfamilies of the order (below the *Hominidae*) as given by Elliot.⁴ The first relationship which presented itself was that between the systematic positions of the specimens from which the hairs were taken, and the diameters of the hair shafts. Fig. 4 graphically records this relationship.

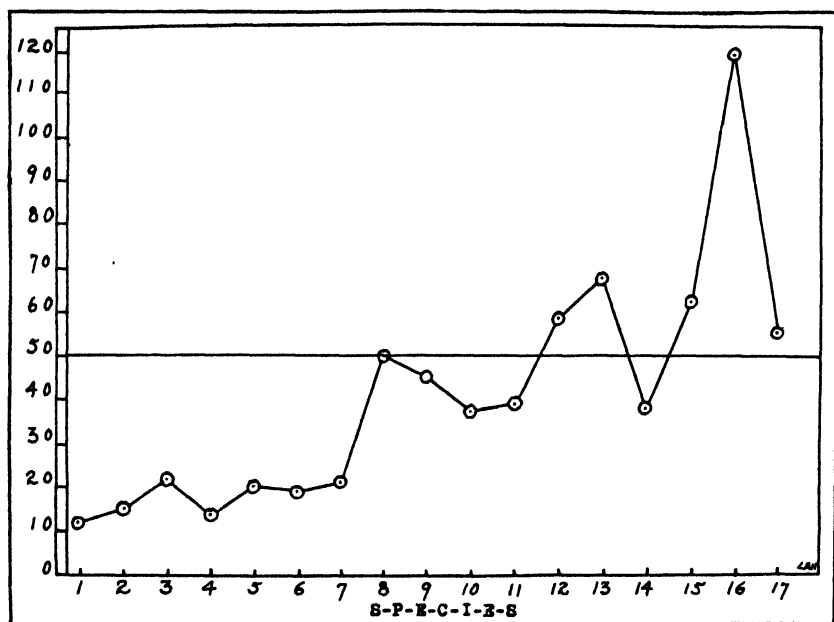


FIG. 4. The relationship between the diameters of the hair shafts of seventeen species of *Primates* and the morphological complexity of the species. Along the ordinate are given the hair shaft diameters in microns, and along the abscissa are enumerated the species in the order of morphological complexity.

It was noted, moreover, that an increase in the diameter of the hair shaft was accompanied by a decrease in the width of the free surface of the cuticular scales, i.e., in its free proximo-distal diameter (F, Fig. 2). A numerical expression of the relation between the free prox-

⁴ Elliot, D. G., "A review of the *Primates*," Monograph, Am. Mus. Nat. Hist., 1912.

imo-distal diameter of the cuticular scales, and the hair-shafts on which they occur was devised, and termed the *scale index*. This is arrived at by dividing the free proximo-distal diameter of the cuticular scales by the diameter of the hair-shaft, and expressing the result in decimal form. Where D is the diameter of the hair-shaft, F the free proximo-distal diameter of the scales, and S the scale index:

$$\frac{D}{F} = S.$$

Computations of the scale indices of the seventeen species of *Primate* hairs mentioned gave the data for plotting the graph shown in Fig. 5.

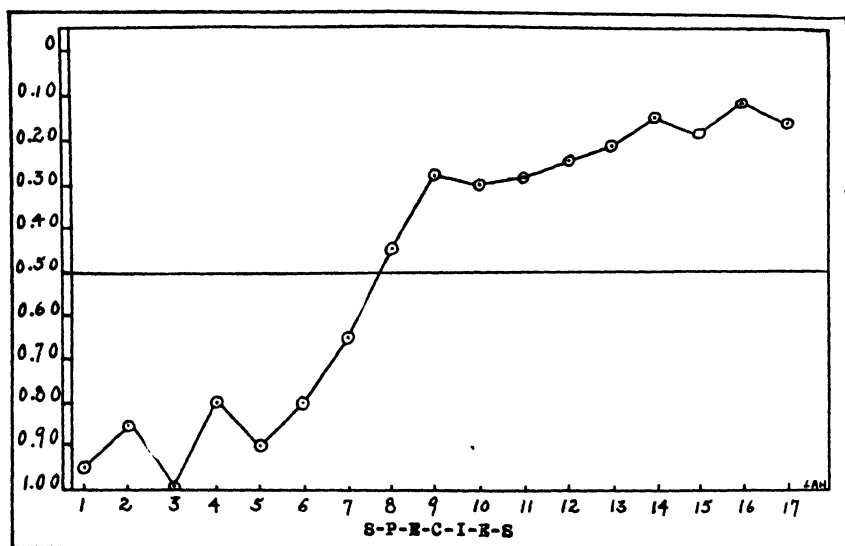


FIG. 5. The relationship between the scale index (see Fig. 2) of the hairs of seventeen species of *Primates*, and the morphological complexity of the species. Along the ordinate are given the scale indices, and along the abscissa are enumerated the species in the order of morphological complexity.

An examination of the medullas of these same specimens of hairs revealed the fact that the increase in the diameter of the hair-shafts was accompanied by a change in medulla form, from the discontinuous type of medulla to the fragmental type, as shown in Fig. 6. The fact that

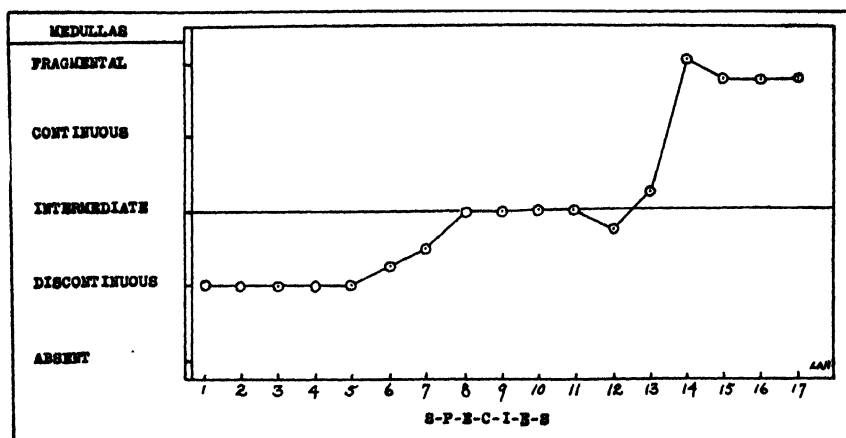


FIG. 6. The relationship between the medulla types (see Fig. 3) in the hairs of seventeen species of *Primates*, and the morphological complexity of the species. Along the ordinate are given the medulla types, and along the abscissa are enumerated the species in the order of morphological complexity.

this comparative study of the hairs of so limited a number of species of mammals, taken quite at random, showed such relationships, indicated that perhaps similar relationships existed among mammals in general, and incited the observer to more inclusive examinations. Accordingly, 190 samples of dorsal under hair, from as many species of mammals (representing all the existing orders except the *Cetacea*) were examined for the status of their scale and medulla elements.

Computations of the scale indices of these hairs and the measurements of their diameters gave the data shown in Fig. 7.

In this figure the diameters of the hair-shafts examined are plotted along the abscissa, and the scale indices along the ordinate. Here again the same relationship between scale index and shaft diameter, as was noted in the *Primate* hairs, became apparent. Since the scale index also denotes, in general, the form of the scale, this relationship between scale index and shaft diameter is interesting and significant, inasmuch as it implies that the forms of the cuticular scales of mammals bear relation, not to the groups to which the species have been assigned, but

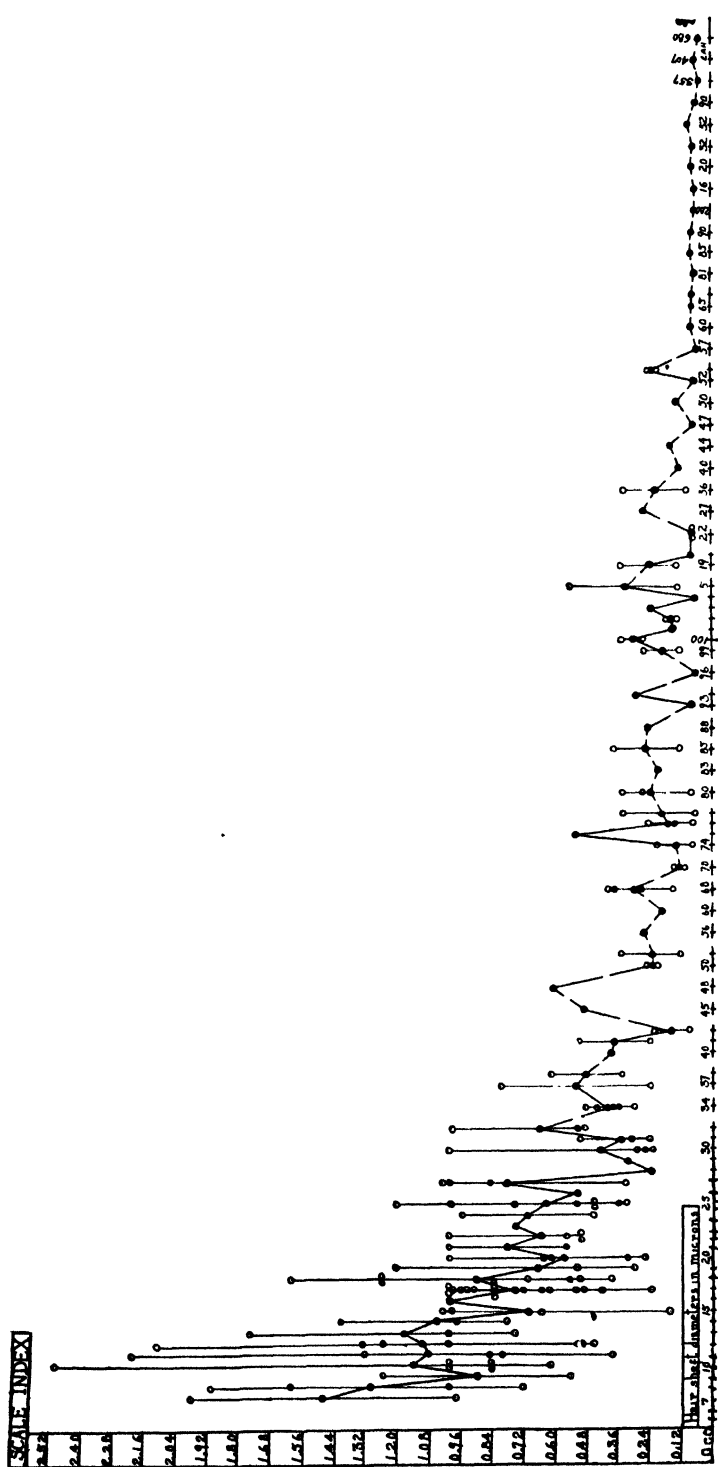


FIG. 7. The relationship between scale index and hair shaft diameter, as shown by the examination of the hair of 190 species of mammals, representing all the existing orders except the Cetacea. Clear circles represent hair samples from the different species examined; filled circles the averages, which are placed on vertical lines indicating the range of scale index. *E.g.*, of hairs 25 microns in diameter, eight samples (from eight different species) were examined, with a scale index range from 0.33 to 1.20. The averages of the indexes for each group are connected.

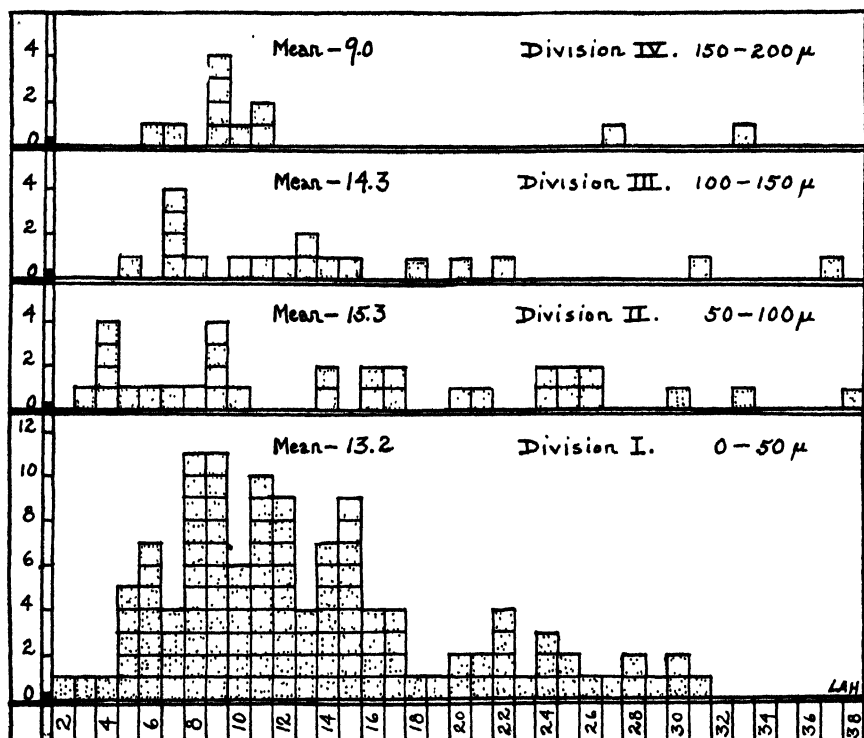


FIG. 8. Lengths of the free proximo-distal diameters of the cuticular scales, plotted against the frequency of occurrence. The micron-length-groups are arranged along the abscissa; the frequency of the occurrence of the examples in each group along the ordinate. The 178 hair-shafts examined (from as many species of mammals) are separated into four groups, or divisions:—Division I contains hair-shafts from 0 to 50 microns in diameter; Division II those from 50 to 100 microns; Division III those from 100 to 150 microns; and Division IV those from 150 to 200 microns. The mean length of scale for each division (*i.e.*, mean proximo-distal diameter).

to the diameter of the hairs which they bear (Plate I). That even one individual may bear upon its body hairs of different and widely separated structural groups was shown in the writer's studies of the hairs of the *Ornithorhynchus anatinus* and the *Tachyglossus hystrix* (1).

Fig. 8⁵ shows the frequency of the occurrence of various cuticular scale lengths (*i.e.*, the proximo-distal diameters), in microns, arranged in groups or divisions. The mean lengths of the scales of the four divisions are much

⁵ The writer is much indebted to Professor W. J. Crozier, of Rutgers College, for his aid in the preparation of this figure.

alike, and the scale lengths are distributed with similar regularity in each of the divisions.

Next, a survey of the medullas of the hairs of 200 species of mammals, representing all the existing orders except the *Cetacea*, yielded the information contained in Table I, with respect to the relationship between medulla-form and hair-shaft diameter. Five different groups of hairs were recognized, according to the types of medullas they contained. Such a grouping showed that as a rule the finer hairs (*i.e.*, those of small shaft diameter) contained no medullas, or either the discontinuous or intermediate varieties; while the coarser hairs bore the continuous or fragmental varieties (see Fig. 3). Only a meagre number of exceptions to this rule were found, as

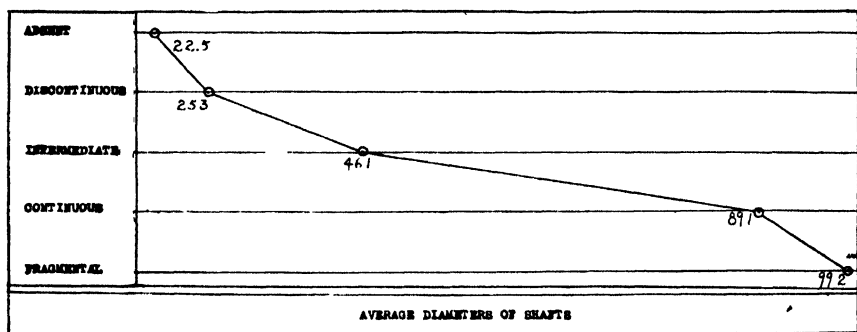


FIG. 9. To show the relation of hair-shaft diameter to type of medulla, a graphic presentation of the material is contained in Table No. 1. Along the ordinate are given the medulla types (see Fig. —), and along the abscissa the average diameters of the hair shafts exhibiting these five different types.

the table shows. Fig. 9 represents in graphic form the results of the study of the distribution of medulla-forms. Here, again, the interest lies in the implication that medulla-form, like scale-form, is related not to natural group, but to hair-shaft diameter. Medulla-form may vary in the hairs even of an individual, as in the case of the *Ornithorhynchus anatinus* and *Tachyglossus hystrix* (1).

An interesting fact brought out by the study of scale types is that with the finer hairs there is a much greater range in the scale index than with the coarser. That is,

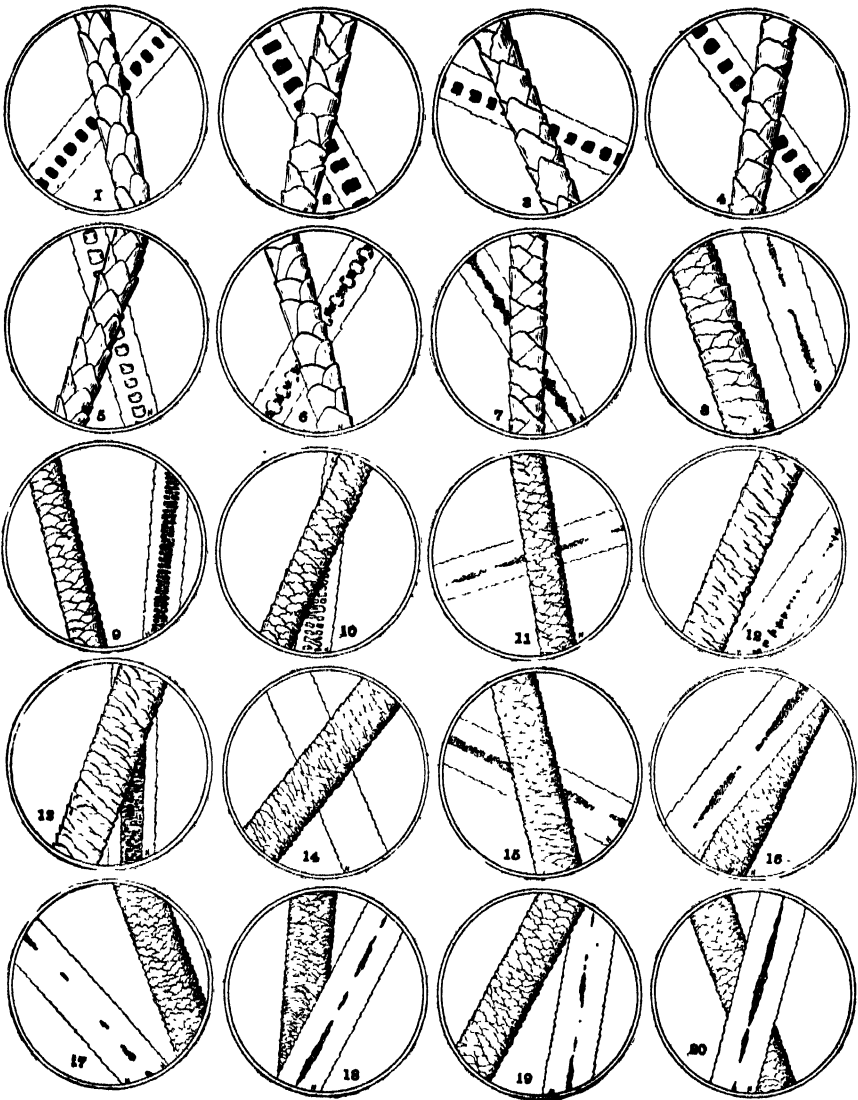


PLATE I

The first sixteen specimens are of dorsal hair, taken from as many species of *Primates*, arranged in the order of the morphological complexity, and representing the nine families and ten subfamilies of the order as given by Elliot (footnote 4). The last four specimens are of human head-hair, from individuals representing four races of mankind. Each figure depicts the portions of two hair-shafts; one prepared to show the cuticular scales, the other to show the medulla. The micrographs were made from the middle portion of the hair (i.e., midway from the base to the tip of the hair-shaft). The numbers following the name of each species in the list below are the diameters of the hair-shafts in microns.

fine hairs may possess relatively very large scales (as in the Golden Mole, *Amblysomus corriae*), or relatively small ones (as in the Flying Squirrel, *Sciuropterus volucella*); while, with the coarser hairs (those, *e.g.*, of a diameter greater than 50 microns) the scales are relatively uniformly small. A large scale index (above 1.80, let us say) usually denotes the elongate or acuminate variety of imbricate scale; or the coronal scale. A medium scale index (about 0.50 to 0.75) denotes, as a rule, some of the larger varieties of the ovate, crenate or flattened scale; while an index below 0.35 usually denotes the smaller variety of either the crenate or the flattened forms (Figs. 1 to 20, Plate I).

FIG. 1. Aye aye (*Chiromys madagascariensis*)—11

FIG. 2. Tarsier (*Tarsius fuscus*)—15

FIG. 3. Potto (*Perodicticus ibcanus*)—22

FIG. 4. Galago (*Galago demidoffi*)—14

FIG. 5. Ruffed Lemur (*Lemur varius*)—20

FIG. 6. Sifaka (*Propithecus coronatus*)—18

FIG. 7. Guenon (*Cercopithecus patas*)—21

FIG. 8. Howler (*Alouatta palliata inconnans*)—50

FIG. 9. Squirrel Monkey (*Chrysothrix sciurea*)—45

FIG. 10. Aotus (*Aotus scner*)—37

FIG. 11. Geoffroy's Spider Monkey (*Atelcs geoffroyi*)—38

FIG. 12. Heuglin's Baboon (*Papio doguera heuglinii*)—58

FIG. 13. Proboscis Monkey (*Nasalis larvatus*)—67

FIG. 14. Hoolock Gibbon (*Hylobates hoolock*)—37

FIG. 15. Gorilla (*Gorilla gorilla*)—62

FIG. 16. Schweinfurth's Chimpanzee (*Pan schweinfurthii*)—118

FIG. 17. Bushman of South Africa (Negro Race)

FIG. 18. Chinese (Mongolian Race)

FIG. 19. Peruvian mummy, of cir. 200 A. D. (American Race)

FIG. 20. English (Caucasian Race)

} 50 to 65

A microscopic study of some fifty-odd specimens of human head-hair, representing all the existing grand divisions of the races of mankind, showed that there were no very well-marked variations in either scale- or medulla-form.⁶ In general, the coarser the hair, the finer

⁶ Further work, tentatively outlined, and covering a greater range of samples, must be done before any definite pronouncement can be ventured regarding the scale and medulla relationships in the hairs of the *Hominidae*. There seem to be, however, considerable variations in the pigment granules

It is rather remarkable, although variations are not uncommon, to find a hair-shaft of this meagre diameter clothed with scales of the flattened type, and of so low an index. The majority of hairs between 10 and 20 microns of the mammals, below the *Hominidae*, that were examined possessed ovate scales, with an average index of 0.90. As a rule, the flattened type of scale, of the index presented by this lanugo, was encountered generally upon hairs of from 50 to 100 microns in diameter. No medulla could be discerned.

SUMMARY

In the specimens of mammal hairs examined:

(1) Scale-form (as expressed by the scale index, a mathematical expression of the relationship between the free proximo-distal diameter of the scales and the diameter of the hair-shaft) bore relation not to the natural group to which any given species belonged, but to the diameter of the hair-shaft. In other words, the coarser the hair the finer the scales, or the magnitudes of the free proximo-distal diameters of the cuticular scales and the diameters of the hair-shafts varied inversely.

(2) The medulla-form varied with the diameters of the hair-shafts, and not with natural groups of mammals, in a definite way.

(3) Hence, given the diameter of a hair-shaft, and regardless of the species from which it was derived, it should be possible to locate it in its proper medulla-form, or scale-form group, approximately.

(4) It is inferred that the relationships between scale-form (as expressed by the scale index), medulla-form and hair-shaft diameter, which have been found in the series of samples examined in this study, obtain also among mammals in general.

(5) From the results of previous studies of mammal hairs, as well as from added results from this present one, it can still be said, however, that specific differences of sufficient appreciable magnitude exist, commonly, to aid in identifying the species of mammal from which a given hair sample was obtained.

SHORTER ARTICLES AND DISCUSSION

THE SIMILARITY OF AGE VITALITY IN INVERTEBRATES AND MAN BASED ON PROFESSOR RAYMOND PEARL'S DATA

DURING recent years Professor Raymond Pearl has published a considerable amount of data regarding the length of life of flies and has constructed life tables from them.¹ The resemblance of these life tables to those of man suggested that it might be worth while applying some further tests to his figures.

Some time ago I devised and published the series of formulae by which the expectation of life in man could be easily calculated by means of the use of a standard population, if the death-rates at certain groups of ages were known. These formulae were based upon the numerous life tables constructed in England and Wales ranging from the life table referring to the very unhealthy district of Manchester Township to that of the healthiest of the country districts. Later, the method was much improved. The standard population now chosen decreases in a simple arithmetical progression as age increases, as was long ago suggested by De Moivre. In practice, the population between 0-5 years is assumed to be 16,000, that between 5-10 years 15,000 and so on. Thus between 25 and 35 years it numbers 21,000, between 35 and 45 years 17,000, while above 75 years it is reduced to 1,000. The death-rates at the groups of ages just described, obtained from the statistics, are applied to this population, and the number of deaths that would occur in this standard population with these death-rates above each age obtained. From these values, the expectation at each age can be at once calculated. The method will be better understood by considering an example. The example selected refers to the long-winged male *Drosophila*, for which if its 100 days of life be taken equal to a 100 years of life in man, the distribution of survivors falls well within the range of human variation. The death-rates were calculated for the age periods 0-5 days, five-daily periods after that to 25 days, thereafter by ten-daily periods to 75 days and lastly the death-rate above 75 days.

¹ Pearl, R., "Medical Biometry and Statistics," p. 177.

The method of working is shown in Table I. In the first column the standard population is given; in the second column the absolute death-rates as calculated; in the third column the sums of the deaths above each age—thus the lowest figure in the column is obtained by multiplying the death-rate in the second column by 1,000. The next figure above it in the column is obtained by adding this to 5,000 multiplied by the death-rate between 65 and 75 years, the figure above by adding to this sum 9,000, multiplied by the death-rate between 55 and 65 years, and so on to the top of the column. Denoting these sums by D_x and the expectations by E_x we have the relationship,

$$\frac{1000}{E_x} = m D_x + c$$

or

$$E_x = \frac{1000}{m D_x + c}$$

The two constants m and c calculated by means of the five best life tables for England and Wales for each age and for both sexes are given in Table II. The figures in column 4 which give the expectations are obtained by the use of the formula and the values of the constants m and c . These are now to be compared with the figures in column 5, which give the expectations of life as calculated by Professor Pearl in the usual manner. It will be noticed that the correspondence is very close except at birth, when the formula derived from man gives 5 days less life

TABLE I
ILLUSTRATION OF THE METHOD

Age	Standard population	Death-rates	Number of deaths D_x	Age	Expectation calculated E_x	Expectation actual
0-5 ..	16,000	.0094	3694.5	0	36.0	41.0
5-10 ..	15,000	.0112	3544.1	5	39.1	38.5
10-15 ..	14,000	.0107	3376.1	10	35.2	35.4
15-20 ..	13,000	.0135	3226.3	15	32.5	32.2
20-25 ..	12,000	.0147	3019.6	20	29.6	29.1
25-35 ..	21,000	.0180	2843.2	25	26.5	26.2
35-45 ..	17,000	.0331	2465.2	35	20.5	20.7
45-55 ...	13,000	.0513	1902.5	45	15.9	16.1
55-65 ..	9,000	.0679	1238.2	55	12.2	12.4
65-75 ..	5,000	.0867	582.0	65	9.4	9.5
75 + ...	1,000	.1370	132.0	75	7.4	7.3

than the experiments. This is to be expected, as there is no infantile mortality among the *Drosophila*.

The subject has been extended and applied to others of Professor Pearl's observations. The comparison of the calculations made by the two methods is shown in Table III. Very close correspondence is shown to exist for the long-winged *Drosophila* of the female sex. Dr. Pearl also gives a life table of a rotifer *Proales* reduced from the data of Dr. Bessie Noyes, adjusting the life of the worm, which is about 10 days, to the 100 years of human life. When the deaths in a standard population are calculated and the formulae applied in the method described, again a close correspondence is obtained.

With regard to a wild *Drosophila*, which has a shorter life extending at most to about 75 days, before applying the method, the data have been adjusted to correspond to a longer life, three days of the flies' life corresponding with four years of that of man. It will be seen from the table that again the fit is exceedingly close. It thus seems that for certain invertebrates the law of age-ing which is derived from man can be applied, though these organisms functionate in a quite different manner.

The question now arises: Is this a universal phenomenon or not? This can only partly be answered from Professor Pearl's data. When observations on the short-winged *Drosophila* and on one of the mutations² are examined, not so close a corre-

TABLE II

GIVING THE CONSTANTS REQUIRED TO CALCULATE THE LIFE TABLE DEATH-RATES FROM THE NUMBER OF DEATHS

Age	<i>Males</i>		<i>Females</i>	
	m	c	m	c
0-5	0.0047986	10.052	0.0046168	10.604
5-10	0.0036317	12.756	0.0039159	12.460
10-15	0.0044503	13.210	0.0043761	13.395
15-20	0.0050789	14.303	0.0052281	14.202
20-25	0.0061455	15.254	0.0063082	15.132
25-35	0.0076268	16.204	0.0077535	16.154
35-45	0.012322	18.546	0.012239	18.759
45-55	0.021530	22.017	0.020931	22.606
55-65	0.044486	26.720	0.043032	27.667
65-75	0.127236	31.954	0.121408	34.123
75 +	1.003635	2.823	0.876036	20.901

² Gonzalez, Bienvenido Maria, "Experimental studies on the duration of life," AMER. NAT., Vol. LVII, No. 651, p. 296.

TABLE III
EXPECTATIONS DEDUCED FROM THE DATA (A), AND CALCULATED FROM THE FORMULA OF TABLE II (B)

	Long winged <i>Drosophila</i> ³				Rotifer <i>Proclita</i> ⁴		Wild <i>Drosophila</i> , Both sexes		Mutations bpr asp		Short-winged <i>Drosophila</i> Females		Manchester Township 1881-90 Males		England and Wales, last quarter, 1918 Females	
	Males		Females		(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
	(1)	(2)	(3)	(4)												
0	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
5	41.0	36.0	35.6	35.1	67.9	65.8	34.8	35.7	17.7	19.5	14.0	16.4	40.53	40.46	38.4	42.8
10	38.5	39.1	32.4	32.8	62.9	62.1	31.6	32.5	16.6	18.3	13.0	15.6	37.47	36.62	35.0	39.2
15	35.4	35.2	29.2	29.5	57.9	56.5	28.7	29.8	15.1	16.6	12.1	14.4	33.56	33.24	32.3	36.1
20	32.2	32.5	26.2	26.5	52.9	51.6	26.5	27.3	14.2	15.2	11.3	13.4	29.61	29.61	30.4	33.5
25	29.1	30.3	23.3	23.8	48.0	47.1	23.5	24.3	12.5	12.6	10.7	12.4	26.00	26.11	28.9	29.5
35	26.2	26.5	18.3	18.6	38.3	37.7	18.5	18.7	10.3	10.7	8.7	9.1	20.01	20.09	23.2	23.4
45	20.7	20.5	14.3	14.5	29.4	28.7	12.8	13.1	8.5	8.6	7.6	7.5	14.93	15.17	17.1	17.2
55	16.1	15.9	11.5	11.2	21.1	20.7	10.0	9.9	6.1	5.8	6.2	5.7	10.96	10.92	11.1	11.4
65	12.4	12.2	8.7	8.2	14.8	14.0	8.1	7.5	5.2	5.0	4.7	4.3	7.48	7.53	6.7	7.0
75	9.5	9.4	6.8	6.0	9.7	9.4	7.4	7.2					4.74	4.65		
85	7.3	6.5														

In this table A denotes the actual figures, B those obtained by the formulae.

The age in the first column is in all cases the corresponding human age.

³ Pearl, R., and Parker, S. L., "Experimental studies on the duration of life," AMER. NAT., Vol. LV, No. 641, p. 494.

⁴ Pearl, R., _____

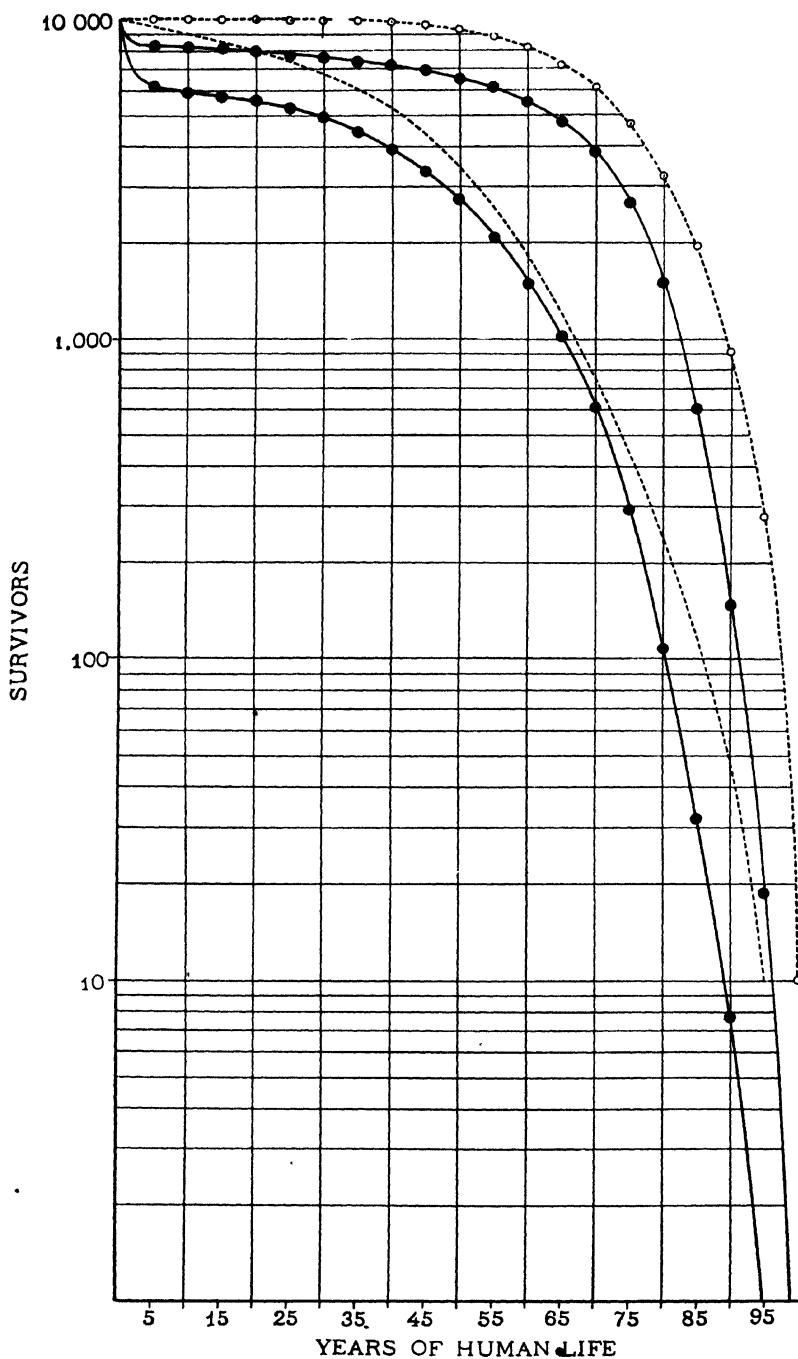
⁵ Gonzalez, B. M., "Experimental studies on the duration of life," AMER. NAT., Vol. LVII, No. 651, p. 296.

⁶ *Ibid.*, p. 303.

⁷ Pearl, R., and Parker, S. L., "Experimental studies on the duration of life," AMER. NAT., Vol. LV, No. 641, p. 497.

spondence is found. The respective results for these are given in columns 11 and 12 and columns 9 and 10 of Table III. It will be seen that above the age corresponding to 35 years the fit is very close and that below this age the formulae based on human life tables in the case of the short-winged *Drosophila* and of the mutations give about one and one half to two days greater expectation. The formula, therefore, fails in this region. A possible reason for this will appear on considering the diagram. In this diagram, the logarithms of the number of survivors in two of the most diverse English life tables, namely, the healthy districts life table for 1881-90 and Manchester Township for the same period, are shown by continuous lines. One dotted line shows the number of survivors for the long-winged *Drosophila*. This curve lies in the zone for which the formulae hold except in the first few days of life. With regard to the rotifer *Proales*, the curve lies outside the curve of the healthy district life table. In this instance an extrapolation is justified by its results, which perhaps might be expected from the close resemblance of the form of the two curves. With regard, however, to the short-winged *Drosophila*, the formulae only hold above the age corresponding to 35 years in man and below that the formulae give an excess. It can not be said that this necessarily means that the laws of life which apply to man may not apply even here because there is no evidence of what occurs with regard to men living in more unhealthy conditions than the Manchester Township. It might be possible that the hyperbolas of the formulae no longer hold beyond the Manchester limit and that a term depending on the second power of the deaths would require to be added. This, however, does not seem likely. The Manchester Township life table was not used in the calculation of the formulae, yet, as may be seen in columns 13 and 14 of Table III, the correspondence between the values of the expectations given in the life table and those calculated by the formulae are very close. An extrapolation ranging considerably beyond the data on which the formulae were calculated is thus found to be justified by the results.

I would suggest that it is possible that some epidemic prevailing among the shorter-winged flies gives rise to the high death-rates between the period corresponding to 10 and 35 years in man. The form of the curve of survivors given by Professor Pearl suggests this. In the last quarter of 1918, a great epidemic



This diagram gives the logarithms of the number of survivors at each age for man and two invertebrates. The upper continuous line refers to the healthy districts of England, 1881-1890, the lower line to the Township of Manchester for the same 10 years. Nearly all the life tables regarding England lie within the zone delimited. The dotted line which lies almost wholly within this zone refers to the long-winged *Drosophila* and the dotted line lying outside refers to the rotifer *Proales*.

of influenza occurred in which the chief excess in mortality was found to be between the ages of 15 and 35 years. Making a life table for the female sex, as on account of the war, the male population can not be estimated, it is found that exactly the same discrepancy between the two sets of calculations found above is obtained. In columns 15 and 16 of Table III, the expectations found by direct calculation are compared with those given by the formulae. Again it is seen that above 35 years of age the concordance is very close, while below this direct calculation gives expectations of life three to four years less than those given by the formulae, corresponding exactly to the one and one half to two days' difference found in the case of the short-winged *Drosophila*.

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NOTE ON DR. JOHN BROWNLEE'S PAPER ON AGE VITALITY¹

It is a great satisfaction to have the independent confirmation, reached by a somewhat different method, which Dr. Brownlee's paper affords, of the results and conclusions regarding the fundamental similarity of the laws of mortality in *Drosophila* and man, which have been published from this laboratory at various times during the past two years.² We have shown by the employment of a method somewhat simpler, but, so far as I can see, in no respect less precise than that of Dr. Brownlee that when the biologically equivalent life spans of man and wild type *Drosophila* (our Line 107, cf. Pearl and Parker, *loc. cit.*, 1924) are compared, age being measured in centiles of the life span, the life table constants for the two cases became so similar as to be practically identical. We have used for the comparison the survivorship or l_x function of the life table. Dr. Brownlee uses chiefly the expectation of life function e_x . But as there is, from the mathematical nature of the case, a complete and perfect correlation between the corresponding l_x 's and the e_x 's of the same life

¹ Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 105.

² Cf., particularly Pearl, R., *AMER. NAT.*, Vol. 56, pp. 398-405, 1922; Pearl and Doering, *Science*, Vol. 57, pp. 209-212, 1923; Pearl, R., *Poultry Science*, Vol. 3, pp. 1-10, 1923; Pearl and Parker, *AMER. NAT.*, Vol. 58, pp. 71-82, 1924.

table, since both are fundamentally derived from the specific death-rates (q_x 's), it makes no particular difference which of the derivative functions one uses as the basis of comparison. It in no wise more cogently or completely demonstrates the fundamental similarity of the laws of mortality in *Drosophila* and man to show that the e_x 's are in close agreement in the two cases than to show that the l_x 's are. What Dr. Brownlee's paper shows that our previous work does not is that his particular method of deriving e_x from a knowledge of d_x in the construction of a life table is just as applicable to mortality data for *Drosophila* as to those for man.

The *essential* point in the methodology of all such comparisons is, of course, the method used to put total life spans which in absolute time duration are widely different upon an equivalent basis. I venture to suggest that all Dr. Brownlee's comparisons would have been quantitatively improved if he had used a somewhat less rough and ready approximation in establishing this datum than the one he did use. Furthermore, there appears to be no biological justification for his assumption that the imaginal life of *Drosophila* and the total postnatal life of man are equivalent.

Dr. Brownlee's suggestion that the widely different form of the life curve of flies bearing the mutant gene *vestigial* from that of either normal wild type *Drosophila* or man is due to an epidemic mortality in vestigials, seems improbable for several reasons. In the first place direct observation of the vestigial flies themselves, in the process of giving rise to their characteristic mortality curve, affords no evidence that they die of an epidemic or indeed any infectious disease. In the second place, we have shown³ that the characteristic vestigial life curve reappears as extracted F_2 vestigials from a cross of wild type and vestigial flies, precisely as though it were a character inherited in a typical Mendelian manner. It would be difficult to suppose that an epidemic disease followed the gene for vestigial around through this complicated genetic pathway. In the third place, we have shown⁴ that under starvation conditions the life curves of vestigial and wild-type flies become practically identical, and like those for the fed wild-type flies, when age is measured in

³ Pearl, Parker and Gonzalez, AMER. NAT., Vol. 57, pp. 153-192, 1923.

⁴ Pearl and Parker, AMER. NAT., Vol. 58, pp. 193-218, 1924; Pearl, R., *Nature*, June 14, 1924.

centiles of the life spans in the two cases. Finally, we have shown, in experiments as yet unpublished, that under at least two different sets of feeding conditions (not starvation) wild type and vestigial life curves can be made nearly identical and of the wild-type (also human) form rather than that of the characteristic vestigial curve on standard food. Altogether, it seems probable that other factors than epidemic disease are responsible for the form of life curve characteristic of vestigial flies on standard food.

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A CASE OF MATERNAL INHERITANCE IN DROSOPHILA

AN increased interest in cases of so-called "maternal inheritance" is evidenced by the recent appearance of several papers. Among these are Uda's article¹ questioning to some extent the frequently cited results of Toyama and Tanaka, the paper of Sturtevant² interpreting in terms of maternal inheritance the data of Boycott and Diver on the inheritance of direction of coiling in *Limnaea* and the general paper of Morgan³ on the heredity of embryonic characters. It was therefore believed that it might be of value to publish at this stage a preliminary note concerning a peculiar lethal recently arisen whose expression apparently is dependent upon the genetic make-up of the mother. A *bona-fide* case of maternal inheritance in *Drosophila*, besides being of general value, would offer exceptional opportunity in the way of analysis.*

The mutant appeared in slightly inbred material of *Drosophila melanogaster* which was concerned in an experiment designed to measure the rate of origin of lethal factors in the X-chromosome. The original culture produced one female and 96 males. This greatly decreased proportion of females in the lethal strain is

¹ Uda, Hajime, 1923, *Genetics*, 8, pp. 322-335.

² Sturtevant, A. H., 1923, *Science*, LVIII, pp. 269-270.

³ Morgan, T. H., 1924, *Scientific Monthly*, XVIII, pp. 5-17.

* Since the above was written an article by Warren has appeared which indicates that the size of the egg of *Drosophila melanogaster* depends upon the genetic constitution of the mother rather than upon the constitution of the zygote itself. This is to be expected since the size of the egg is said to be fixed before the entrance of the spermatozoon. Warren, Don C., 1924, *Genetics*, 9, pp. 41-69.

typical; however, there is some variation in the proportion, for although many cultures produce very few females or none, in others the sex ratio approaches equality. The type of sex ratio in which the females are decidedly less numerous is extremely uncommon in *Drosophila*, for departures from equality in the sex ratio of these flies are usually due to an elimination of males by lethal or semi-lethal genes in the X-chromosomes. However, 31.5° C. decreases the proportion of females.⁴ Moreover, sex-limited mutations, *e.g.*, truncate, are known, the expression of which is more extreme in the female than in the male and which may have a corresponding differential effect on the viability of the two sexes. A striking example of this type of mutation was reported by D. H. Thompson⁵ at the 1920 meeting of the American Society of Zoologists. A sex-limited, sex-linked recessive lethal had been found that killed all females homozygous for it and caused no lethal effect in the males, but an erect position of the wings. A different mechanism apparently was at work in the culture reported in 1910 by Quackenbush;⁶ this gave 135 males and no females, a ratio much like those given in the present case. This family was not further analyzed, but the results of Sturtevant⁷ on the crosses between *D. simulans* and *D. melanogaster* make it practically certain that the sex ratio obtained by Quackenbush was due to a cross between these two species, which were at that time not distinguished. The cross of *D. simulans* female by *D. melanogaster* male—but not the reciprocal cross—gives a greatly lowered proportion of females. As will be seen from the following account, the results of outcrosses of the lethal-bearing strain in the present case have features in common with those of this species cross.

The mode of inheritance of the new lethal is of more interest than the reduced proportion of females produced. The effect is transmitted by both sexes. If females from the lethal strain are crossed to males of other stocks, the offspring may give the lethal ratio; but if the reciprocal cross of a lethal-bearing male by a female of another stock is made, the immediate offspring do not give a lethal ratio. The crosses may be so arranged that the progenies have in the two cases the same genetic constitution;

⁴ Mann, M. C., 1923, *Jour. Exp. Zool.*, 38, pp. 233-244.

⁵ Thompson, D. H., 1921, *Anat. Rec.*, 20, p. 215; an abstract.

⁶ Quackenbush, L. S., 1910, *Science*, XXXII, pp. 183-185.

⁷ Sturtevant, A. H., 1920, *Genetics*, 5, pp. 488-500.

the only difference being, then, that when the lethal is introduced from the mother most of the daughters die, but when it is introduced from the father they live. In other words, whether a given female lives or dies depends not upon her own genetic composition, aside from the fact of her being a female, but upon that of her mother alone. These crosses as well as others, all to be reported in detail later, indicate "maternal inheritance" of the lethal, as the term is used by Morgan, Sturtevant and others. It is obvious that the greatest difference between the female zygotes of the two crosses lies in the origin of their cytoplasm. The cytoplasm of the eggs from the female of the lethal strain has been so affected (probably before maturation, since such females apparently must be homozygous to give the effect) that the combination of lethal cytoplasm plus two X-chromosomes plus the autosomes is very much less viable than the combination of non-lethal cytoplasm plus two X-chromosomes plus the autosomes, or than lethal (or non-lethal) cytoplasm plus X plus Y plus the autosomes. Whether the Y of the male, whose sisters die, is responsible *per se* for his survival can not at present be definitely stated. That the majority of the female zygotes from a mother of the lethal strain have actually died (or have not been formed), instead of having been "transformed" into males, is suggested by the absence of any intersexual manifestations; it is possibly also indicated by the low yields from culture bottles giving a lethal count. This problem and others are being studied by cytological methods.

When the lethal is crossed to curly stock (the curly gene is a second-chromosomal dominant with a recessive lethal effect, and is linked to certain non-crossover genes⁸) and when the offspring are inbred, the curly females of succeeding generations, irrespective of the origin of their cytoplasm, never give lethal ratios among their immediate offspring, but the non-curly females may do so. That is to say, the hereditary base of the lethal effect involves at least one recessive gene, and this gene is located in the second chromosome. There is, of course, no discrepancy between the fact that specific chromosomal genes are responsible for the effect and the implication that these genes leave their imprint upon the cytoplasm of the individual before the maturation of the egg giving rise to that individual.

⁸ Ward, Lenore, 1923, *Genetics*, 8, pp. 276-300.

The theoretical application of the work to the problem of species incompatibilities may briefly be mentioned. The action of the lethal, in killing all or practically all the females of certain cultures, prevents the reproduction of those individuals. But of greater importance in this connection is the situation as regards the mothers of the dead females; the mothers are partially prevented from producing viable offspring, and are therefore partially physiologically isolated. As in the cross between *D. simulans* and *D. melanogaster*, so here in outcrosses of the lethal to non-lethal lines, the female offspring are rarely able to develop if the egg cytoplasm has been supplied by one of the lines, but develop readily if the cytoplasm has been derived from the other line (*i.e.*, in the reciprocal cross). The present case differs from the species cross in that it is possible to analyze the genetic basis of the cytoplasmic influence (which is not possible in the species cross because the hybrids are sterile), and in that evidence exists concerning the origin of this genetic basis by mutation from the parent stock. We have here, then, a condition approaching in some respects reproductive incompatibility, and this condition is dependent upon a specific genetic constitution. The suggestion is not offered that the lethal exhibits phenomena of complete physiological isolation, but that certain analogies to such isolation are shown in its behavior which may be of value in considerations of the genetic basis for the reproductive incompatibilities presented by related species and in determining how such incompatibilities arise.

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MUTANT FORMS OF MATTHIOLA RESULTING FROM NON-DISJUNCTION^{1 2}

FOUR of the mutant forms of stock described by Frost³ (Large-leaved, Smooth-leaved, Crenate-leaved and Slender) have always produced, when selfed, a mixture of normal and mutant-type progeny. Three of these forms, Large, Crenate and Slender,

¹ Paper No. 116, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

² Cytological work done mainly by the junior author, in the Division of Genetics at Berkeley.

³ Frost, Howard B. "Mutation in *Matthiola*," Univ. California Publ. Agric. Sci. 2: 81-190. 1919.

have recently been found to possess an extra chromosome in addition to the 7 pairs normal to the variety from which they arose. Occasional non-disjunction has been observed in the normal form, and this doubtless accounts for the origin of gametes with 8 chromosomes. Union of one of these with a normal gamete would produce a 15-chromosome mutant. About 2 to 5 per cent. of the progeny of normal plants have been classed as mutants.

When Slender and Large are intercrossed, four classes appear in their progeny—Normal, Large, Slender and Large Slender. Pollen mother cells of Large Slender at the first metaphase contain 9 chromosomes (7 bivalents and 2 univalents), and the root-tip cells contain 16 chromosomes. While it can not be said, on the basis of the observations now available, that the two odd chromosomes of Large Slender never pair, they certainly do so rather infrequently if at all. If Large and Slender were due to ordinary gene differences combined with trisomy in the same chromosome group, their normal progeny should be different. Since their normal progeny seem to be identical, it is probable that at least these two of the mutant forms are due to non-disjunction of chromosomes belonging to different normal pairs. This conclusion is strengthened by the great somatic dissimilarity of the two types; and in view of the situation presented in the final paragraph below, this further evidence is needed. Large Crenate also has two odd chromosomes; one of these resembles the odd chromosome of Crenate in form, and is unlike those of Large and Slender.

Plants of these four mutant types are generally more or less deficient in vigor as compared with normal plants, and statistical evidence of selective elimination of the former at germination has been secured.³ There is also evidence of selective elimination of gametes carrying an extra chromosome, somewhat as in the case of simple trisomic forms in other genera. In *Datura*, Blakeslee⁴ (summary) reports that the pollen of the trisomic Globe type has transmitted the Globe character to only about 2 per cent. of the progeny in back crosses to normal, while the ovules have transmitted it to about 26 per cent. In the 15-chromosome *Oenotheras*, de Vries and Boedijn⁵ report that the tri-

⁴ Blakeslee, Albert F. "The Globe mutant in the jimson-weed (*Datura Stramonium*).'' *Genetics*, 6: 241-264. 1921.

⁵ De Vries, Hugo, and Boedijn, K. "On the distribution of mutant characters among the chromosomes of *Oenothera Lamarckiana*.'' *Genetics*, 8: 233-238.

somic types are not reproduced at all by means of their pollen. Crenate *Matthiola*³ is transmitted by the pollen to some 5 per cent. of the progeny, or about one fifth of the total percentage to which it is transmitted in selfing. Slender *Matthiola* differs decidedly from all these forms, since in back crosses the pollen has transmitted the mutant type to nearly one fifth of the progeny, and the ovules to about one third of the progeny, while the proportion of Slender from selfing is slightly higher still. In general, the four mutant types of *Matthiola* mentioned above have been transmitted, in selfing, to from 25 to 50 per cent. of the progeny; and it is plain that differential viability of both gametes and zygotes has contributed to this result.

It may be assumed that tetrasomic zygotes are usually non-viable. As with Globe *Datura*,⁶ however, trisomic Slender parents give occasional progeny which manifest the mutant characteristics in an extreme degree. These Extreme-slender plants are very small and feeble. Some of their pollen mother cells contain 8 pairs of chromosomes; others, 7 pairs and 2 unpaired chromosomes. Cells with 8 pairs reduce normally, but in those containing 2 univalents the unpaired elements, like the single extra element in the 15-chromosome plants, divide in the first division and assort at random in the second. The very meager breeding data from one supposedly Extreme-slender parent³ agree with the cytological results in that some normal progeny occurred, with an excess of Slender. There is no evidence that the odd chromosome of either Large or Slender unites characteristically with any of the 7 pairs of chromosomes.

The Slender type is associated with singleness of flowers, in a manner suggestive of linkage. The double-flowering plants, which are always completely sterile, are evidently pure recessives (*dd*).⁷ The singles of a double-producing race are then *Dd*, and carry a pollen lethal which sterilizes all *D* pollen: selfed normal single parents usually give slightly more than 50 per cent. of double progeny. Slender gives a large excess of singles among its Slender progeny, and (except in the cross normal ♀ x Slender ♂) a large excess of doubles among its normal progeny. Slender as seed parent, whether selfed or pollinated by normal, gives an abnormally high proportion of total doubles

⁶ Blakeslee, Albert F. "Variations in *Datura* due to changes in chromosome number." *AM. NAT.* 56: 16-31. 1922.

⁷ Frost, Howard B. "The inheritance of doubleness in *Matthiola* and *Petunia*. I. The hypotheses." *AM. NAT.* 49: 623-636. 1915.

(about two thirds), which is presumably due to selective elimination of Slender. The general trend of the observed ratios is explained if we assume that all trisomic Slender singles adequately tested have been *Ddd* (*D* being dominant over *dd*), and that much selective elimination of the Slender type occurs. The breeding data, however, show a marked deficiency in the normal single class when Slender is seed parent, and the ratios are otherwise different from those expected. The basic gametic ratio from random reduction would be $2Dd : 1dd : 1D : 2d$, the *D* pollen being non-functional as usual. For pollination of Slender by normal (pollen all *d*) the expected ratio (without selective elimination) is, therefore, 2 Slender single : 1 Slender double : 1 normal single : 2 normal double. The observed ratio is at present 56 : 21 : 22 : 137, and larger numbers from selfing show a similar departure from the expected ratio among the normals. The situation is evidently complex, and further consideration of hypotheses must be reserved for a more detailed report of this work to appear elsewhere.

A plant has been described⁵ which had several Slender single branches, while the other branches and the upper main axis were normal double; if this plant was originally *Ddd* (Slender single), the bud variation is readily explained by early loss of the *D* chromosome in a cell of the apical meristem.

The fact that Crenate also shows genetic association with single³ seems to be a serious difficulty in the way of any consistent and plausible general scheme for these forms. It is suggestive of the "varieties" of some of the trisomic forms of *Datura*,⁶ although Slender and Crenate are not very similar morphologically.

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LUTEAL CELLS AND SEXUAL DIMORPHISM OF FEATHERING IN WILD BIRDS

BORING and Morgan (1918)¹ have shown that hen-feathering in the male Sebright Bantam is associated with the presence of

⁵ Frost, Howard B. "An apparent case of somatic segregation involving two linked factors." *AM. NAT.* 55: 461-464. 1921.

¹ Boring, A. M., and Morgan, T. H., 1918, "Luteal cells and hen-feathering." *Journ. Gen. Physiol.* 1.

luteal cells in the testis. Since luteal cells are also a constant element in the ovary of the domestic fowl it has been suggested that hen-feathering in female fowls and in the male Sebright is due to a hormone secreted by the luteal cells which suppresses the development of cock feathers.

These conclusions suggested an investigation of the question whether or not there is a correlation between the sexual dimorphism in the feathering of wild birds and the presence or absence of luteal cells in the gonads, and also an investigation of the relation of the structure of the gonads to the seasonal variation of plumage. It seems advisable to give at this time a brief statement concerning the first question, inasmuch as considerable time will be required to satisfactorily work out the latter problem.

In order to test out the possible relation of the presence of luteal cells to feather coloring, a histological study has been made of the gonads of both sexes of a number of wild birds which for convenience have been grouped as follows: (1) Those in which the female shows the higher degree of coloration, such as the northern phalaropé; (2) those in which there is no marked difference in the coloring of the two sexes, such as the killdeer, spotted sandpiper, steller jay and water ouzel; and (3) those with the male possessing the more brilliant plumage, represented by the robin, western bluebird, flicker, bob white, California quail, rusty blackbird and China pheasant.

The observations recorded here were made on the gonads of birds taken in late winter, spring and early summer. Immediately after the birds were shot their reproductive organs were removed and put into Bouin's fluid. The sections were for the most part stained with Delafield's haematoxylin.

In an earlier report² it was shown that as regards the phalarope no evidence was obtained indicating that luteal cells in any way influence the difference in feathering in the sexes. In no case were any luteal cells found in the testes of the phalarope, while sections of the ovaries showed them in considerable abundance. Since, in this instance, the female is the more brilliantly colored, the evidence that luteal cells secrete a hormone having a suppressing influence on feather-coloring is negative.

A study of the gonads of the birds in the second group showed luteal cells present in all the ovaries, but with the possible excep-

² Yocom, H. B., "Luteal cells in the gonad of the phalarope," *Biol. Bull.* 44, March, 1924.

tion of the testes of spotted sandpiper no cells were found in the testes which at all resembled luteal cells. In the testes of the sandpiper there were large cells located between the seminiferous tubules, resembling somewhat the characteristically grouped luteal cells of the ovary. Whether or not these are actually luteal cells must remain undetermined until another migration period, when more material will be available.

In the gonads of the birds of the third group every ovary studied possessed characteristic packets of luteal cells, but in no case were any such cells found in the testes.

Of the birds thus far studied there is no positive indication that luteal cells are present in the testes, while in all cases they were found in the ovaries. Such evidence would indicate that, for the birds studied, it is not a hormone secreted by luteal cells that has a suppressing influence on the development of color in the feather. Indeed, no evidence is at hand which would warrant any suggestion concerning the function of these characteristic groups of cells in the ovaries of wild birds. Nonidez³ has shown that in the fowls studied by him such cells arise from embryonic sex cords. This, however, does not give us any clue to their function, much less furnish evidence of their controlling influence on the color of feathers.

We must, however, bear in mind the fact, as suggested in the work on the phalarope, that in most fowls feather structure differs in the two sexes, while in the wild birds studied, with the exception of the gallinaceous birds, sexual differences in feathering seem to be due to color rather than to morphological differences of the feathers.

It is possible that a study of the relation of the structure of the gonad to the seasonal variation in feathering may give some evidence leading to an understanding of this perplexing question of the sexual dimorphism of feather color in wild birds.

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³Nonidez, J. F. 1922, "Studies on the gonads of fowls. The origin of the so-called luteal cells in the testis of hen-feathered cocks." *Am. Jour. Anat.*, 31, 109-124, 7 figs. in text.

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